# Nitrogen Acquisition of Lentil (*Lens culinaris* Medic) Under Varied Fertility Treatments, No Tillage Duration and Nitrogen Regimes in Saskatchewan

A Thesis Submitted to the College of Graduate Studies and Research

In Partial Fulfillment of the Requirements

For the Degree of Doctor of Philosophy

In the Department of Plant Sciences

University of Saskatchewan

Saskatoon

By

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# ABSTRACT

High levels of soil nitrogen (N) can interfere with $N_2$ fixation of lentil (*Lens culinaris*) and have variable effects on growth, yield and maturity of this indeterminate crop in Saskatchewan. In a series of field and greenhouse experiments from 2006 to 2008, response of the above-ground biomass (DW), plant N, $N_2$ fixation, yield and days to maturity (DTM) of lentil to different N sources, time of N availability, and also to two lengths of no tillage (NT) treatments were studied.

First, eight cultivars of lentil were grown under three fertility treatments of granular rhizobium inoculant, 50 kg N fertilizer $ha^{-1}$ and a non-treated control in three environment-years at Saskatoon and Indian Head, SK. The fertility treatments, plant N status and $N_2$ fixation did not alter lentil DTM, but weather did. On average, lentil matured 101 and 84 days after seeding with sufficient rain and with drought, respectively. Growth and yield of lentil were identical in the inoculant and the N fertilizer treatments. The N fertilizer treatment occasionally restricted $N_2$ fixation, but N shortage from fixation was compensated via more N uptake from soil. The highest N accumulation in lentil occurred from podding to maturity. By maturity, pod, stem and leaf had 60, 24 and 14% of total dry matter and 78, 9 and 13% of total plant N, respectively. Leaf N concentration, which closely resembled soil and plant N status, was reasonably predicted by SPAD chlorophyll meter observations after pod set.

Yield of five lentil cultivars was tested for the effects of 25-years (long-term no tillage:LN) versus 5-years (short-term no tillage: SN) in the Black Soil Zone at Indian Head, SK in 2006, 2007 and 2008. In the same location, CDC Sedley was grown with four N fertilizer rates under

both LN and SN. Under terminal drought in 2006, average DW, N content and yield of the lentil cultivars in SN were greater than in LN. In this year, 60 kg N fertilizer $ha^{-1}$ reduced the yield difference of CDC Sedley between SN and LN. Lentil yield was identical or tended to be greater in LN than in SN with more rain in 2007 and 2008 that possibly prolonged N mineralization and N uptake. With the greenhouse study, applying N fertilizer from flowering to podding and flowering to maturity increased DW, N content and yield, and delayed maturity of lentil compared to lentil relying on $N_2$ fixation. Later flowering of one cultivar or greater $N_2$ fixation in one soil medium diminished the variation of inoculated lentil with the post-flowering N treatments, suggesting N fixation could supply lentil N requirement.

Large-seeded cultivars produced greater yield than the small-seeded cultivars across environments in the fertility treatment study. Cultivar CDC Milestone produced comparable yield to high-yielding cultivars CDC Plato and CDC Greenland, but matured earlier. This cultivar showed promising results under both cool-wet and drought conditions. In contrast, CDC Sedley had lower $N_2$ fixation and HI values across the experiments. In the Black Soil Zone, growth and yield CDC Milestone and CDC Robin was greater than other cultivars, likely because of more $N_2$ fixation and efficient translocation of carbon and N to seed.

Overall, results of this thesis do not support the application of N fertilizer for inducing early maturity in lentil. Soil inoculation with commercial strains is suggested for Saskatchewan cropping systems. Applying N fertilizer is not required, unless soil test results suggest otherwise. In places like Indian Head, SK, cultivars with greater $N_2$ fixation and higher HI would benefit the short growing season, cool temperature and high soil N content.

# ACKNOWLEDGMENTS

I have received infinite support from my supervisor, Dr. Rosalind Bueckert during my PhD study at the University of Saskatchewan. I owe thanks for her continuous encouragement, advice and support throughout the PhD program. In addition to the assistances on designing experiments, analyzing data and interpreting the results, Dr. Bueckert spent an enormous amount of time helping me to improve my academic writing. She has been a wonderful academic supervisor, and a supportive friend to my family and me. Thank you Ros.

I am greatly thankful to my advisory committee for their continued supervision throughout my PhD project: Dr. Guy Lafond for helping me with understanding the cropping systems of western Canada, and directed me toward more efficient thesis and manuscript writings; Dr. Jeff Schoenau for helping me with experimental designs, interpreting the results and preparing manuscripts; Dr. Bert Vandenberg and Dr. Bruce Coulman for proving helpful advice during advisory meetings.

I would like to appreciate helps from the crop physiology lab technician, Janet Pritchard, for helping in data collection and lab measurements. Great helps in seeding and taking care of the experimental plots by Research Farm staff in Indian Head and Field Lab crew in Saskatoon is appreciated. I wish to express my thanks to NSERC & the Saskatchewan Pulse Growers for providing funding for the research, and to the "Ministry of Science and Technology" in Iran for the scholarship.

Last but not least, I must acknowledge all supports from my wife, Marzieh, who sacrificed much of herself to provide a peaceful environment for everyone at home while I was busy at the school.

## Dedication

This work is dedicated to my beloved wife Marzieh,
my son Pooya and my daughter Paris
who dedicated their comfort to my work

برای :

مرضیه، پویا و پریس

# Contents

## List of tables

## List of figures

## List of abbreviations

| **Acronym** | **Explanation** |
|---|---|
| **C** | Carbon |
| **C:N** | Carbon: nitrogen ratio, used to determine the quality of crop residues |
| **CDC** | Crop Development Center, Saskatoon, SK |
| **CT** | Conventional tillage: seed-bed preparation through burying crop residues prior to seeding the next crop |
| **cv** | Cultivar |
| **DAS** | Days after seeding |
| **DTM** | Days from seeding to maturity |
| **DW** | Dry weight: total plant dry weight at a given growth stage |
| **FAO** | Food and Agriculture Organization, United Nations |
| **GD** | Goodale Research Farm, located 16 km from the University of Saskatchewan main campus |
| **GDD** | Growing degree days: cumulated degrees above a base value during the entire or for any period during plant growth |
| **HI** | Harvest index: the proportion of seed weight to total plant biomass at physiological maturity |
| **IH** | Indian Head, Saskatchewan, refers to trials conducted close to the town of Indian Head, SK |
| **LN** | Long-term no tillage practices: refers to a no-till treatment, was tested in the thesis with duration of >25 years |
| **N** | Nitrogen |
| **N%** | Nitrogen concentration in plant or seed, weight basis |
| **$NH_4^+$** | Ammonia |
| **NN** | Neural network |
| **$NO_3^-$** | Nitrate |

| | |
|---|---|
| **NT** | No-tillage: Growing crops without disturbing the soil by tillage, it is also known as zero-tillage |
| **SKA** | Skarsgard Research Farm, located at about 22 km from the University of Saskatchewan main campus |
| **SLN** | Specific leaf nitrogen: the ratio of leaf nitrogen content to leaf area |
| **SLW** | Specific leaf weight: the ratio of leaf weight to leaf area |
| **SN** | Short-term no tillage practices: refers to a no-till treatment, was tested in the thesis with duration of >5 years |
| **$T_b$** | Base temperature: minimum temperature required for plant growth. Although $T_b$ may differs as plant grows or in different environments, it is assumed similar for the entire growth season or varied environments, when it is used to calculate GDD |
| **MP** | Mid-pod: in this thesis, mid-pod is referred to >1 pod plant in 80% of plants in plot |
| **PM** | Physiological maturity: Crops reach their maximum dry weight. In this thesis, physiological maturity was defined as the minimum of 80% yellow canopy in each plot |
| **KWT** | 1000-seed weight |
| **NI** | Inoculated treatment in the greenhouse study |
| **NF** | Applying nitrogen fertilizer until flowering, in the greenhouse study |
| **NP** | Applying nitrogen fertilizer until pod formation, in the greenhouse study |
| **NM** | Applying nitrogen fertilizer until maturity, in the greenhouse study |
| **VDW** | Plant vegetative biomass, calculated as the accumulated biomass in stem and leaf during the flowering to maturity in the greenhouse study |
| **M1** | Soil medium #1in the greenhouse study: autoclaved Sunshine mix number 4 |
| **M2** | Soil medium #2 in the greenhouse study: equal mixture of field soil, river sand and Sunshine mix number 4 |
| ***%Ndfa*** | The proportion of atmospheric N to the total plant N, estimated as the proportion of $^{\delta 15}$N of $N_2$-fixing crops to a non-fixing reference crop |
| **$^{15}$N (‰) or $\delta^{15}$N** | The proportion of concentration (percentage) of $^{15}$N in a sample compared to its concentration in atmosphere |

# 1.0 Introduction

Canada is the world's largest exporter of lentil (*Lens culinaris* Medic.), and Saskatchewan leads the country in lentil production. Cultivation of this valuable crop has substantially increased in Saskatchewan due to intensified and diversified cropping systems and, more importantly, due to economic benefits. For example, the lentil price has been increased from \$353 in 1998 to \$735 metric tonne$^{-1}$ in 2009 (FAO, 2010). In 2009, Saskatchewan farmers harvested 1,480,000 metric tonne lentil from 953,000 ha. However, lack of adaptability of lentil to the variable environment of western Canada challenges its yield potential. A range of 797 to 1397 kg seed ha$^{-1}$, recorded during 1998 to 2009 in Saskatchewan (Government of Saskatchewan, 2010) indicates the instability of lentil yield from year to year.

In addition to unpredictable weather conditions, indeterminate growth habit of lentil is associated with yearly variation in yield. As an indeterminate crop, lentil yield is formed under late season conditions, and late season weather is highly variable and unpredictable in the Canadian prairies. Lack of a determinate cultivar has limited lentil producers to narrow practical options for controlling late season growth, delayed maturity and harvest. Because nitrogen (N) is the most abundant mineral element in plants, this thesis research assumed that manipulation of N availability to lentil can change plant N status and, thereby, affect maturity, harvest index (HI) and yield of lentil.

## 1.1 Lentil maturity and $N_2$ fixation

Lentil is an indeterminate crop, with continuous post-flowering vegetative growth due to plant genetics (Benlloch et al., 2007). As indicated by Kusmenoglu and Muehlbauer (1998a), lentil breeding has targeted a shortened life cycle because lentil is largely grown in restricted conditions by drought (Shrestha et al., 2006), or early frost (Kusmenoglu and Muehlbauer, 1998b). In the northern Great Plains, limited heat accumulation and risk of early frost damage (Miller et al., 2002) can be more critical for lentil and chickpea. Risk of later maturity is increased with late season rainfall and high soil N. Such conditions stimulate crop vegetative growth over seed production (McKenzie and Hill, 1995; Wallace et al., 1990; Gooding et al., 2003).

A determinate cultivar in lentil has yet to be developed. Among different possibilities, manipulation of plant N status via changing soil available N may induce early maturity in lentil. Plant maturity is triggered by growth inhibitor hormones, which are controlled by light quality or environmental stress such as water and nutrient deficiency (Biswal and Biswal, 1984; Thomas and Stoddart, 1980). Nitrogen deficiency may also be associated with the concentration of maturity induction hormones (Pastori et al., 2003). However, lentil obtains up to 80% of its N requirement via atmospheric N fixation, and is less likely to face N deficiency during growth (Kurdali et al., 1997; van Kessel, 1994), but N sufficiency may play a role in delaying maturity.

Manipulating soil N content and rhizobia population can impact $N_2$ fixation, and is extensively described in the literature (Bremer et al., 1989; Gan et al., 2005; Kyei-Boahen et al., 2002; Osborne and Riedell, 2006; Salvagiotti et al., 2008). In the semi-arid environment of Saskatchewan, applying N fertilizer in non-inoculated chickpea reduced days to maturity of this indeterminate crop by 15 days (Gan et al., 2009a). Although $N_2$ fixation of chickpea was not measured, the authors claimed that the applied N fertilizer inhibited $N_2$ fixation, lowered late season plant N content and accelerated maturity. The strategy of maturity induction by N fertilizer must carefully consider that lowered $N_2$ fixation will increase production costs of pulses that normally rely on $N_2$ fixation and also of non-pulses, which benefit from N left in the rotation by pulse crops (Walley et al., 2007).

The idea of early maturity induction in lentil via lowered $N_2$ fixation was the main frame of the first experiment of this thesis. This idea was divided into three hypotheses below, by applying three fertility treatments of inoculant rhizobia, 50 kg N $ha^{-1}$ and a non-treated control to lentil.

1. *Nitrogen fertilizer will reduce $N_2$ fixation of lentil in symbiosis with soil indigenous rhizobia. The lowered $N_2$ fixation will reduce plant N content and, thereby, induce earlier maturity, compared to the inoculated lentil.*
2. *Different N fertility treatments will influence time of the availability of N to the plant. More available N to the fertilized lentil at early growth will increase pre-flowering dry weight (DW); whereas post-flowering growth will be promoted in the inoculated lentil. This variability will impact partitioning of DW and N within lentil plants.*
3. *Response of different lentil cultivars to the N fertility treatments will be different. Available cultivars in Saskatchewan differ in days to maturity, seed size and therefore seed N demands. Their $N_2$ fixation ability and potential for biomass and N accumulation will also vary. Less response to N fertility treatments is expected in low yield potential cultivars with small biomass.*

**1.2 No tillage duration and lentil yield**

Since the early 1980s, no tillage (NT) has been widely practiced in western Canada, where seed is sown directly into stubble of previous crops in rotation (Schillinger and Papendick, 2008). Long-term continuous NT has noticeably improved the quality of agricultural soils via improved soil moisture, soil organic matter and soil N. Ongoing studies in different parts of the American Great Plains has demonstrated that long-term NT practices can noticeably increase soil organic and inorganic N (Liang et al., 2004; Malhi and Kutcher, 2007; Schoenau et al., 2008).

Large amounts of N accumulated during the course of NT (Schoenau et al., 2008), but may not necessarily translate to higher yield. Field crops are seen to increase vegetative biomass at the expense of seed yield in the presence of water and N (Gooding et al., 2003). Association of soil N with plant growth in legumes will differ from non $N_2$ fixing crops. Lack of response to added N (Cowell et al., 1989), or greater growth in response to more N, the later via inhibited $N_2$ fixation, is expected from legume crops grown under NT systems. Lentil, as an indeterminate crop, may invest more carbohydrate in vegetative biomass than in seed yield in presence of more available N in NT (Bremer, 1989). In addition, days to maturity of lentil may be extended in response to more available N in soil (Saskatchewan Pulse Growers, 2000).

Due to accumulating N during the course of NT, lowered HI and delayed maturity is expected from lentil as the NT system ages. Additional N fertilizer may increase vegetative growth, or it may lower $N_2$ fixation and induce early maturity. Two other hypotheses were developed to test the effect of NT duration on growth and yield of several lentil cultivars, and the effect of additional N fertilizer on a late maturing cultivar of lentil, CDC Sedley.

1. *High soil N mineralization due to long-term no tillage will increase plant DW, N content and yield and extend the period of seeding to maturity. Lentil grown in the long-term NT will have lower HI compared to the one in the short-term NT. Response of varied cultivars to the duration of NT will differ because cultivars vary in biomass, N accumulation and seed yield.*
2. *Response of lentil to N fertilizer will be reduced with more years spent under no-till, because of more available N in long-term than in short-term NT.*

## 1.4 Post-flowering N fertilizer and lentil yield

Substantial seed N concentration in pulse crops, ranging from 18 to 30% (Sinclair and de Wit, 1975), suggests that insufficient N supply towards maturity will reduce yield. Maximum $N_2$ fixation in pulses occurs prior to maximum N requirements (van Kessel, 1994). Under this circumstance, remobilization of N from vegetative organs to seeds is an effective mechanism for supplying seeds with enough N (Masoni, et al., 2007). However, the quantity of remobilized N to seeds is highly affected by both the environment and crop.

In irrigated conditions, lentil remobilized less N from leaves and stems to seeds than what is observed in wheat (van Kessel, 1994). In addition, nodules still compete with seeds for carbohydrate (Wallace et al., 1990; Salvagiotti et al., 2008). The ratio of lentil N to DW declined as plants grew (van Kessel, 1994). Dilution of N due to large biomass close to maturity is common among crops and may indicate lack of sufficient N for maximized yield. The 6$^{th}$ hypothesis of this thesis was developed based on the idea that lentil $N_2$ fixation, N uptake and N remobilization are not adequate to maximize yield. The hypothesis was tested by applying N fertilizer to lentil after flowering and podding stages and was compared with an inoculated control treatment:

*Application of N during the reproductive growth phase will reduce competition between seed and other plant organs for N, and will increase leaf longevity, days to maturity and yield. Response of different cultivars to the post-flowering N application will be different.*

## 1.3 Leaf N prediction by SPAD

Monitoring leaf N concentration throughout the growing season may indicate plant N status. Such information can be used in split N application management or for developing models to predict yield quality and quantity (Le Bail et al., 2005). However, measurement of leaf N concentration is time consuming and expensive. Alternatively, leaf chlorophyll content, measured by the Soil Plant Analysis Development (SPAD) chlorophyll meter, has been used as an indirect estimate of leaf N status in field crops (Esfahani et al., 2008; Le Bail et al., 2005; Shafagh-Kolvanagh et al., 2008; Yamamoto et al., 2002). If leaf N concentration in lentil is linked to SPAD readings, fast and economical estimate of leaf and plant N status should be possible. I hypothesized that:

*Leaf N status will represent the entire plant N status in response to varied environments and N availability. Leaf chlorophyll content, estimated by the SPAD chlorophyll meter, will provide accurate estimation and prediction of leaf N concentration.*

## 1.5 Objectives

The research in this thesis aimed at investigating the value of applying N fertilizer, as a replacement for rhizobia inoculant and $N_2$ fixation, for enhancing yield and early maturity in lentil. Due to limited literature on this topic for lentil, growth and N partitioning in a range of cultivars was investigated with various weather and soil N scenarios. In addition, the effect of N deficiency or sufficiency on yield during reproductive growth was investigated.

<u>The specific objectives of this research were:</u>

1. *To assess DW, N accumulation, $N_2$ fixation, HI, seed yield and days to maturity of different cultivars of lentil grown in the field under three N fertility treatments of 50 kg N $ha^{-1}$, inoculated with rhizobia and untreated control in different environments and soil zones of Saskatchewan.*
2. *To measure within-plant partitioning of DW and N in eight cultivars of lentil in response to fertility treatments*
3. *To measure response of lentil growth, N accumulation, yield and days to maturity to length of no-till in the Black Soil Zone of Saskatchewan*
4. *To assess the effect of controlled N application during reproductive growth on DW, N accumulation, $N_2$ fixation, HI, yield and maturity of five cultivars of lentil in a greenhouse study.*
5. *To predict leaf N concentration as an indicator of plant N status using SPAD chlorophyll readings*

# 2.0 Literature review

## 2.1 Lentil

Lentil is one of the oldest grain legume crops grown across a wide geographical range and varying environments around the world. Lentil seeds are rich in protein, carbohydrate some other essential nutrients and they contain more protein than other pulse crops (Muehlbauer et al., 2007); they are consumed as a main dish, side dish or in salad (Yadav et al., 2007). In 2009, worldwide production of lentil was 3,595,177 metric tonnes from 3,637,000 ha (FAO, 2010).

### 2.1.1 Lentil morphology

Lentil is an annual plant with an average height of 25cm (range of 15 to 75cm) and an indeterminate growth habit that allows continuous post-flowering vegetative growth (Tullu et al., 2001; Muehlbauer et al., 2007). Stems are thin in the early-season and get tidier as plants grow (Saxena, 2009). Internode length is short at early growth stages, and internodes extend during crop growth, but new internodes are shorter after flowering (Saxena, 2009). Maximum node number and biomass accumulation occurs around peak flowering to early pod formation (Saxena, 2009; Tullu et al., 2001).

Branches in lentil are categorized as primary (on the main stem), secondary (on the primary branches) and tertiary branches (on the secondary branches). The number of branches per plant varies with plant population density (Wilson and Teare, 1972). On average, main stem, primary and higher order branches carry 18, 52 and 30% of the plant pods, respectively (Erskine and Goodrich, 1991). Seed size in lentil is categorized into two major groups of large-seeded (macrosperma: greater than 50 g per 1,000 seeds) and small-seeded (microsperma: 40 g or less per 1,000 seeds). Lentil has two main market classes, green and red, that are marketed as whole seed or de-hulled and split forms (Saxena, 2009).

### 2.1.2 Lentil maturity

Lentil is an indeterminate crop and its vegetative growth continues after flowering for a period of 10 to 40 days (Saxena, 2009). The number of days from sowing to maturity is affected by temperature, rainfall and sowing date, and ranges from 75 to 180 days (Saxena, 2009). In the northern Great Plains of North America, lentil matures 88 to 101 days after sowing (Miller et al., 2003). In the study of Gan et al. (2005) lentil matured 94 days after sowing. In two different locations of Pullman, WA (the adapted region) and Reading, UK (the non-adapted region), average crop duration was 91 and 146 days, respectively (Whitehead et al., 2000). Although longer days to maturity may increase biomass and yield (Thomson et al., 1997; Whitehead et al., 2000), it may lower HI and be suggested to frost damage in short growing area (Saskatchewan Pulse Growers, 2000).

Lentil requires 944 to 1270 heat units (GDD) from seeding to maturity to complete its growth. This heat requirement is generally achieved in most parts of the Great Plains (Miller et al., 2002). Cooler temperatures may reduce yield by reducing harvest index. In field pea, cooler day/night temperature during seed filling lowered seed N concentration (Larmure et al., 2005). In low rainfall conditions of Australia, early flowering and pod-set helped lentil to escape terminal drought, but a short pre-flowering growth period decreased lentil yield (Thomson et al., 1997).

On average, lentil flowered 31 to 106 days after sowing, and matured 26 to 66 days after flowering in a study with 287 core collection genotypes and 15 registered cultivars in Pullman, Washington (Tullu et al., 2001). In drier conditions, early flowering cultivars tend to increase HI by increasing the length of pod-filling period and by avoiding late-season drought (Erskine et al., 1989; Silim et al., 2008). In WA and ID, each day of delayed flowering and delayed maturity increased straw yield and decreased seed yield (Kusmenoglu and Muehlbauer, 1998a).

**2.2. Indeterminate growth**

As an indeterminate crop, longer days to maturity in lentil may affect the yield by increasing the number of high order branches (secondary and tertiary branches) which carry fewer pods than main stem and primary branches. However, Erskine and Goodrich (1991) did not find a significant relationship between seed yield and the number of primary, secondary and tertiary branches in the Mediterranean climate of Syria. They found that a longer period to maturity increased plant height and significantly reduced seed yield, indicating that post-flowering growth enlarged branch length instead of increasing branch number. These associations may differ in areas with late-season rainfall. Determinate cultivars in lentil and chickpea have yet to be developed; hence, higher plant density, N management and desiccant application are some of the strategies used to accelerate maturity (Gan et al., 2009a; Yadav et al., 2010).

The flexible growth habit of indeterminate cultivars in variable environments is advantageous for plant survival and yield. Indeterminate cultivars of soybean (*Glycine max* L. Merr) yielded more than determinate cultivars in late-planted or in a double-cropping system, because they produced more stems and seed per plant and had a longer seed-fill period than determinate cultivars (Sohedjie and Weaver, 1995). In contrast, terminal drought forced lentil to mature and enhanced the partitioning of assimilate to seed by reducing sink-source competition (Silim et al., 2008).

With sufficient moisture, nitrogen stimulates plant vegetative growth by affecting source-sink relationships. In potato, long photoperiod, high temperature and high available N caused poor assimilation allocation to tubers and delayed maturity (Kooman and Rabbinge, 1996). Similar results were obtained in pea grown under low temperature conditions (Larmure et al., 2005).

With indeterminate soybean, exhausting the accumulated N from vegetative organs determined physiological maturity (Munier-Jolain et al., 1994). Applying 150 kg N fertilizer $ha^{-1}$ stimulated branch length and reduced the yield of indeterminate soybean through lodging; whereas yield in a semi-determinate cultivar benefited from N fertilizer (Wallace et al., 1990).

Long days in northern latitudes increases photosynthesis but also influences other physiological aspects of plant growth. Rate of diurnal starch breakdown in soybean leaves was impacted by day length (Hewitt et al., 1985). In this study starch breakdown was faster in long-day plants than in short-day plants, indicating the possible effect of day length on carbohydrate translocation and HI. Lentil is a facultative long day plant, with flowering induced by days over 14 hours (Roberts, et al., 1986). Since flowering of indeterminate plants continues during reproductive growth, continuous long-days in northern latitudes will influence the duration of the reproductive phase. The literature lacks data on duration of flowering in lentil and the phenological controlling factors of daylength and temperature. In Saskatoon, at 52°N latitude, day length remains longer than 14 hrs until August 25th.

### 2.3 Maturity and N remobilization

Leaf N remobilization and leaf senescence, the latter initiated by seed formation, triggers the maturity in plants. Nitrogen remobilization reduces leaf photosynthesis and thereby lowers $N_2$ fixation because root nodules require carbohydrate supply from the shoot. In irrigated plots in SK, only 17 kg N $ha^{-1}$ was translocated from leaves to seed of lentil by physiological maturity (van Kessel, 1994). Nitrogen removal from vegetative organs became significant in the dry conditions of Syria where lentil translocated 30 to 74 kg N $ha^{-1}$ from the above ground biomass to seed (Kurdali et al., 1997). Early senescence of soybean cultivars had lower $N_2$ fixation (assessed by acetylene reduction) compared to delayed senescence (Abu-Shakra et al., 1978).

## 2.4 Nitrogen and yield

Nitrogen requirements are greater than other elements in field crops. But the association of N and yield varies with plant cultivar and environment. In soybean, 100 kg N fertilizer $ha^{-1}$ resulted in greater seed size and HI for a determinate cultivar, but these effects were reversed with an indeterminate cultivar (Gutiérrez-Boem et al., 2004). In contrast to soybean, lentil produced taller plants, more branches, more pods and higher seed yield in response to N fertilizer in India (Verma and Kalar, 1981).

In addition to environment and growth type effects, $N_2$ fixation of legume crops is also affected by fertilizer. Under favorable conditions, lentil can obtain most of its N requirement through $N_2$ fixation (Kurdali et al., 1997; van Kessel, 1994). This ability is strongly inhibited by drought (Cowell et al., 1989), lack of efficient rhizobia (Gan et al., 2005) and high levels of inorganic N in soil (Bremer et al., 1989). Inadequate N toward the end of season, when $N_2$ fixation is lowered from less assimilate availability, may fail to further increase yield, or even decrease yield. Application of N to inoculated soybean at flower initiation resulted in greater seed yield, dry matter and plant N content compared to a treatment which had received only 120 mg N $pot^{-1}$ prior to seeding (Isfan, 1991), suggesting an inhibited $N_2$ fixation due to the N fertilizer application at seeding.

Response of legume crops to post-flowering N applications varies by growth duration i.e. the period of seeding to maturity, and $N_2$ fixation ability (Freeborn et al., 2001). Application of 25 kg $ha^{-1}$ starter N plus 50 kg $ha^{-1}$ of post-flowering N increased green pod and seed yield of soybean by 44% without reducing $N_2$ fixation (Yinbo et al., 1997). Conversely, in dryland soybean, N application at pod elongation or seed-fill did not increase yield (Schmitt et al., 2001). Maximum $N_2$ fixation in lentil occurs at early flowering under dry conditions (Kurdali et al., 1997), and up to pod formation under irrigation (van Kessel, 1994). In irrigated lentil in Saskatchewan (van Kessel, 1994), the smaller increasing rate of N compared to the increasing rate of DW during reproductive growth suggested insufficient plant N for maximizing yield. Greater plant N content after flowering would increase the yield.

### 2.5 No tillage

Early concepts of soil conservation have developed into strategies like reduced-tillage, minimum tillage and no tillage (NT) around the world (Triplett and Dick, 2008). Along with extraordinary benefits to the environment, conservation tillage is attractive to farmers. Minimized soil disturbance can also lower production costs by diminishing tillage and plowing expenses, lowering nutrient leaching and fertilizer costs (Griffith et al., 1988), and protecting agricultural soil from salinization (Foltz et al., 1995). Improved soil moisture via NT has lowered the need for frequent fallow, and has gradually intensified the traditional wheat-fallow rotations to more diverse, more sustainable and more profitable cropping systems (Zentner et al., 2004). According to Lafond and Clayton (2010), long-term NT has extensively benefited the environment of western Canada by protecting soil from wind and water erosion. Nevertheless, most of the early proposed concerns of NT, such as cool soil temperature and field infestation with weeds and diseases, have not been substantiated. The globally recognized benefits of NT in the North American Great Plains is most likely associated with the specific weather conditions in this region, where drought frequently affects plant growth and yield.

The greatest impact of conservation tillage systems on crop yield is associated with improved moisture conservation. This impact can lower the severity of seasonal drought, support crops for a longer growth period, and produce greater yield. In central Alberta, Wang et al. (2007) measured 10-33 mm greater moisture at 0-60 cm of soil in NT compared to conventionally tilled

(CT) soil. In another study in Alberta, yield in a barley-canola-pea rotation was greater in NT than in CT due to effective moisture conservation (Soon and Arshad, 2004). In thin-black chernozem, greater preserved water resulted in greater response of plants to N and phosphorus (P) fertilizers in NT over CT (Campbell et al., 2001). Greater yield of barley in NT over CT was observed only in drier years (Arshad et al., 1999).

Results of no-till studies indicate that advantages associated with NT are more likely to happen under limited conditions of moisture. In the study of Soon et al. (2008), wheat yielded more in NT over CT only in dry conditions, and the yield was less affected by tillage than by rainfall distribution. The influence of seasonal rainfall and temperature on the benefits of NT has been frequently noted in the literature (Lamb et al., 1987; Lafond et al., 1992; Curran et al., 1992; Doughton et al., 1993; van Kessel and Hartley, 2000; Cutforth, 2002; Perez-Bidegain et al., 2007). In Indian Head, SK, superior yield of spring wheat in NT than CT was recorded in the "sub-optimal" growing conditions of high temperature and low precipitation (Lafond and Derksen, 1996).

In contrast, reduced crop growth via NT, especially at early stages of establishment, is likely. Occasional reduced yield due to NT has been associated with variables like soil structure, nutrient availability, soil temperature, rainfall and temperature variation, crop rotation, and seeding time (Malhi and Nyborg, 1991; Lafond et al., 1992; Malhi and Lemke, 2007). With 12 inches (300 mm) rainfall, wheat, barley and oat produced 5% less yield in NT than in CT in 3 out of 4 years (Schillinger, 2005). This result was explained by less soil moisture capillary activity in CT, which restricted water movement and improved yield by holding more moisture in the soil surface layers. In a long-term (>20 years) tillage study in Montana, NT increased the density of the soil top 10 cm, but soil water content was independent from tillage intensity (Jabro et al., 2009). In Saskatchewan, NT could modify soil pH, but it increased bulk density of the top 5 cm of soil in 3 out of 4 years (Arshad and Gill, 1996). In central Alberta, only the 15 cm upper of NT soil had greater moisture than CT, and barley yielded greater in NT over CT in the 0 kg N treatment only. Lack of response to tillage in the presences of greater N in this study may indicate that N became a limiting factor for plant growth and yield, because sufficient water was available (Malhi and Nyborg, 1992).

In NT systems, soil protection, crop water and conservation, water use efficiency and modified fluctuation of the plant microclimate are all directly associated with the remaining crop residues on the soil surface (Malhi et al., 2007b). Crop residues improve soil water conditions via increased water infiltration rate, soil water holding capacity (Dalal, 1989), soil microbial populations (Halvorson et al., 1999) and soil aggregation (Arshad and Gill, 1996). Soil organic carbon (C) is increased via minimized soil disturbances, proper crop rotation and adequate fertilizer and manure application (Liu et al., 2006). During the course of NT, the concentrations of C and N are increased, while soil pH and soil salinity, which can restrict soil aggregation, are reduced (Lupwayi et al., 1998). In northern British Columbia, greater soil water retention in NT over CT was associated with greater soil organic C (Arshad et al., 1999). In a low-organic matter soil, corn yield in NT steadily increased with time and became similar or exceeded the yield in CT due to improvements in soil conditions (Griffith et al., 1988). In a long-term (11 years) study in southwestern Saskatchewan, Campbell et al. (1996) measured 3 tonnes greater C $ha^{-1}$ in NT than in CT, with the greatest increase in the presence of more residues. Similar results have been found in other studies (Malhi et al., 2001; Malhi et al., 2007b; Kutcher and Malhi, 2010).

Along with increasing soil C, organic N is also increased during the course of NT. In Saskatchewan, total soil C and N as well as soil aggregation were improved by NT, whereas burning crop residues lowered the soil physicochemical properties (Malhi et al., 2007b). Compared to CT, NT had 16 to 40 kg $ha^{-1}$ greater N in three soil zones of Saskatchewan, with the greatest increases in fine sandy loam, silty clay and clay soils (Liang et al., 2004). By employing PRS™ probe technology on soil incubated for two weeks for two fields with long (25-year) and short (5-year) histories of NT duration at Indian Head, Saskatchewan, Schoenau et al. (2008) measured greater N and phosphorus (P) values in the long-term NT soil. They concluded that N shortage due to N immobilization, which may occur at early stages of NT, disappeared as the NT system aged.

Gradually improved soil organic N during the aging of NT or, reduced N by cultivation of natural ecosystems, have both been observed in different locations and climates (Follett and Schimel, 1989; John et al., 2008; Wienhold and Halvorson, 1999; Franzluebbers et al., 1994). Despite increasing total soil N during the course of NT, soil available N may remain unchanged. For instance, potentially mineralizable soil N of NT was higher than CT in only one (Saskatchewan) out of four provinces in Canada (Sharifi et al., 2008). They concluded that greater soil N content may translate to more available N in specific ranges of temperature and rainfall. Contradicting results of soil N cycling due to NT and CT could also be associated with experimental methodology. Soil N content is dramatically reduced by increasing tillage intensity (Follett and Schimel, 1989; Rasmussen and Rohde, 1988).

In addition to direct effects of NT on soil N status, tillage intensity can also change the plant-N associations via altering plant N demand, yield potential and soil N cycle, all of which are associated with soil moisture (Malhi et al., 2001). Soil banding of N fertilizer, common in NT management, lowers the volatilization, denitrification and leaching of N, and noticeably improves N plant use efficiency. However, N leaching due to more available water and better soil infiltration of NT systems may become an issue. In both black and gray soils in Alberta, amounts of $^{15}N$-labeled fertilizer recovered from soil and plant were higher in CT than in NT, suggesting more N leaching from NT than CT (Malhi et al., 1996). Early season N deficiency due to N immobilization, which may be a perceived problem early stage of NT, disappears by greater N cycling as the NT system ages (Malhi et al., 2001; Schoenau et al., 2008). Certain improvements in soil N content together with altered plant-N relationships in NT systems require an adjustment for N fertilizer recommendations that had been previously based on tilled soil (Malhi et al., 2001; McConkey et al., 2002).

### 2.5.3. Soil temperature under NT

Diminished tillage can reduce soil temperature and possibly crop canopy temperature. This modified soil and canopy temperature can produce contradictory effects on yield. Whereas cooled soil may reduce yield of warm season crops in a cool area, it can reduce heat stress and increase yield in a warmer area. Cooler soil temperature under NT systems is critical for warm season crops like soybean and corn. In a 16-year study in southeastern Nebraska, corn yield was lower in NT than in CT, but soybean yield did not respond to the tillage treatments (Wilhelm et al., 2004). In different locations of WI, cooler soil temperature in NT lowered germination and emergence rates and delayed vegetative growth and silking of corn (Carter and Barnett, 1987). The lowered yield by NT was greater in cool than in warm years. At the same location (Arlington, WI), corn yielded less in a cool than in a warm year, but the difference between NT and CT was only significant in the warm year (Azooz et al., 1995). Greater yield potential in warmer years is therefore more likely to be affected by cool soil temperature in NT.

Considering the cold winters and occasional cool summers on the northern Great Plains, field crops may not receive sufficient heat to maximize yield. Under such circumstances, cooler soil in NT can intensify the effect of a cool growing season. In the study of Wang et al. (2007) on a thin black clay loam soil in central Alberta, the surface (both 5 and 10 cm) layer of NT soil was consistently cooler than in CT during the entire growing season. The NT soil saved 10-33 mm more water for the soil depth 0-60 cm. In Manitoba, NT fields had cooler temperatures and more moisture than CT fields when straw was dispersed on the soil surface. As the growing season progressed, the variation in temperature between NT and CT was reduced by an extended crop canopy and stubble breakdown (Gauer et al., 1982).

Crop residues alter soil microclimate via lowered soil temperature and soil radiation absorption, which can reduce the yield in cool areas. In contrast, residues can improve yield via reduced wind velocity and increased snow trapping (Cutforth et al., 2006). In the northern Great Plains in Montana, crop residues increased albedo, reduced wind velocity and boosted accumulated snow compared to bare plots. These effects together improved crop yield because maximum soil temperature during heating days was 10°C cooler in the presence of residues compared to bare soil (Aase and Siddoway, 1980). In the Canadian prairies, lower temperature near the soil surface increased average grain yield of wheat via increased water use efficiency in hot years (Cutforth, 2002).

In Saskatchewan, cool season pulses are dominant, with the exception of warm season common bean (*Phaseolus vulgaris* L.), which recently cultivated in some areas. Most studies from the Canadian prairies emphasize the negligible effects of NT on soil temperature, seed germination and plant emergence (Lafond and Clayton, 2010). In the case of lowered germination rates due to cooler soil temperatures in NT, agronomic practices such as higher seeding rates (Wilhelm et al., 2004) or removing crop residue from seeding rows (Arshada and Azooza, 2003) are suggested to reduce any delaying effect of cool soil temperature on emergence in NT systems. Despite concerns about cool soil temperature effects on seed germination and crop establishment, NT effects via soil and canopy temperature on plant growth and development in cool area needs more investigation.

In North Dakota, growth, DW and N accumulation of field pea responded to tillage, but the response varied by years (Deibert and Utter, 2004). Although the authors failed to indicate whether results were significant, the greatest reduction in DW in NT compared to CT (plow, chisel or disk) were observed in the absence of N fertilizer. Differences of pea DW under varied tillage treatments were associated with rainfall distribution and cumulated GDD. More rain in April and May resulted in negligible differences of DW between tilled and NT plots by flowering. By maturity, late season rainfall produced more plant DW in tilled plots than in NT. In Pennsylvania, CT resulted in 70-110% more emerged weeds compared to NT (Esbenshade et al., 2001).

**2.5.4. Pesticides and NT**

Crop residue can lower weed density efficiently (Anderson, 1999), but the probability of disease and weed infestations are higher due to more moisture in NT systems (Esbenshade et al., 2001). In the study of Arshad et al. (1995) weed population and weed composition tended to increase in response to NT. In another study, NT stimulated foliar disease due to cooler soil surface temperature and greater canopy moisture (Kutcher and Malhi, 2010). In a 5-year study in north-central Saskatchewan, CT practice or burning crop residues slightly reduced the severity of leaf spot diseases in barley and canola, but final yield was greater in NT than in CT due to available water (Kutcher and Malhi, 2010).

The volume of applied pesticides in NT is noticeably greater than in CT, and the greater organic matter content of NT soils can increase retention time of these applied pesticides. Soil organic matter improves during the course of N, and pesticides are bonded to soil organic C and less subjected to biological degradation. However, greater microbial activity under NT systems can compensate for slower degradation rates of chemicals trapped in organic matter. Considering the amounts of applied pesticides and the cold soil conditions during winter on the Canada prairies, one may be concerned about possible undesired residual effects of pesticides in the rotation. In the cold environment of Finland (around 60° north) persistence of several herbicides, including glyphosate, in clay and sandy loam soils demonstrated that glyphosate was preserved until the next cultivation season (Laitinen et al., 2006). In addition, detecting residual glyphosate in deep soil showed more complex mechanisms of glyphosate movement than movement only by leaching. After reviewing almost 100 studies on glyphosate, Borggaard and Gimsing (2008) concluded that glyphosate is less adsorbed by soil organic carbon. Instead, this widely used herbicide is more likely to be held by soil minerals (Al and Fe). Conversely, Landry et al. (2005) measured less glyphosate in water leached from a soil covered by rye-grass (*Lolium multiflorum* Lam) than bare soil, suggesting more glyphosate was absorbed by plant residues. In the study of Weed et al. (1995), late season concentration of herbicides, sprayed on the soil surface, did not significantly vary by tillage intensively.

In a review, Alletto et al. (2010) concluded that pesticide absorption is enhanced under conservation tillage due to greater sorption capacity of crop residues than sorption of soil in tilled plots. The amounts of pesticide retention was correlated to soil organic carbon content, soil moisture, soil temperature and soil acidity, and chances of herbicide leaching in NT was greater than in CT. The greatest leaching of another extensively used herbicide, Atrazine, occurred in high organic matter soil with good aggregation, but leaching was independent from tillage (Montoya et al., 2006). Similarly, tillage intensity did not alter movement of Atrazine and Alachlor in the soil profile in North Dakota (Clay et al., 1998). In five different soils in western Canada, degradation of Flucarbazone was reduced by increasing soil organic carbon and soil dryness (Eliason et al., 2004). Moist soil in reduced tillage systems may influence the effectiveness of herbicide on weeds and crops. Despite similar concentrations of herbicides in soil, corn injuries were greater in a less intensified tillage than in CT (Curran et al., 1992). In soybean, greater insect infestation in NT compared to CT was attributed to insects preferring the higher N crop (Harper et al., 1989).

In Indian Head, SK, lentil in NT yielded 250 kg $ha^{-1}$ less than in CT when subjected to pre-emergence herbicide application (Matus, 1997). In Tennessee, NT and cover crops together increased adsorption of herbicides via improved soil organic matter, but herbicide degradation rate remained unaffected (Brown et al., 1994). Contrasting results in herbicide studies suggested more investigation is needed on the effects of residual herbicides on plant growth and crop yield in cold climates like the northern Great Plains. These studies should address combined effects of rainfall and temperature as well as the quantity of crop residue, plant root system and soil structure.

Residual effects of herbicides on $N_2$ fixation has been also studied on the Prairie. Taylor (2009) could not find negative effects of previously applied herbicides on the ability of rhizobia inoculation or $N_2$ fixation of pea. However, this work suggested that some herbicides restricted early season biomass accumulation and, thereby, plant nodulation. Contrasting results from growth chamber and field studies directed Niina (2008) to conclude that residual effects of the herbicide Flucarbazone-sodium could potentially lower growth and $N_2$ fixation of pea, especially under cold-dry conditions when herbicide degradation was low.

**2.5.4. Nitrogen fixation under NT**

Minimized tillage intensity can increase $N_2$ fixation via improving soil moisture. Also, NT can lower mineralization and nitrification while boosting up immobilization and denitrification of N, all of which improve $N_2$ fixation (van Kessel and Hartley, 2000). Focusing on the northern Great Plains cropping systems, they suggested that $N_2$ fixation of pea, soybean and lentil in NT was 31, 10 and 10% greater when compared to performance in CT. In response to 14 days drought during the growing season, soybean *%Ndfa* and total fixed N in NT was greater than in CT (Harper et al., 1989). Findings at Indian Head, SK demonstrated that $N_2$ fixation of pea and lentil in NT was improved by 10% and 31%, respectively compared to CT (Matus, 1997). Growing soybean in intact soil resulted in more mychorrhiza infection and rhizobia inoculation than growing in a sieved soil (Goss and De Varennes, 2002). Despite similar nodule number in both undisturbed and sieved soils 23 days after seeding, soybean gained four times more nodule weight and fixed more N in the intact soil than in the disturbed soil. In Nebraska, non-symbiotic $N_2$ fixation by *Azotobacter*, in wheat-fallow rotation in NT was twice as much as in plowed soil (Lamb et al., 1987).

In addition to the impact of soil moisture on $N_2$ fixation, immobilized N due to accumulated crop residues in NT could lower readily available nitrate at early season and stimulate nodulation and $N_2$ fixation of legumes (Kristensen et al., 2000). In Germany, delayed N supply in NT than in CT significantly increased $N_2$ fixation of pea; however, final plant N content was equal for both tillage systems (Reiter et al., 2002). In Queensland, Australia, N accumulation of chickpea in NT was 200 kg $ha^{-1}$ greater than CT at maturity, likely due to lowered soil nitrate and more $N_2$

fixation in NT than in CT (Horn et al., 1996). Similarly, chickpea fixed more N after sorghum than after fallow due to a reduction in nitrate which inhibits $N_2$ fixation (Doughton et al., 1993).

In contrast, the gradually increased soil N content during the course of NT may restrict $N_2$ fixation of legume crops (Doughton et al., 1993). Yet plant growth and yield may not necessarily be affected, because the lowered $N_2$ fixation will be compensated by higher soil N availability in NT soils (Malhi et al., 2001). In the Mediterranean climate of Spain, $N_2$ fixation of chickpea and fababean (*Vicia faba* L.) were independent of tillage intensity, but NT plants had less N uptake from soil than CT plants (Lopez-Bellido et al., 2006; López-Bellido et al., 2004). Total N comparison in pea and red clover (*Trifolium pratense* L.) showed that plant growth duration influenced the response of plant N to soil N (Reiter et al., 2002). Field pea with a shorter growing period responded better to N than red-clover, which could uptake more N from NT than CT.

**2.5.5. Pulse crops under NT**

More available water in NT systems has diversified the conventional wheat-fallow rotation of the northern Great Plains with pulse and oilseed crops. Pulses are well known for their $N_2$ fixation ability and their positive contribution to soil N budget (Miller et al., 2002). However, adaptation of pulses to the northern Great Plains has been continually challenged by the uncertain continental climate of the region (Miller et al., 2003). In general, GDD requirements for maturity of lentil, dry pea and chickpea are met in most locations of the Great Plains (Miller et al., 2003). By considering 21 studies in different location-years of the Great Plains and a base temperature of 5ºC, they estimated that lentil requires 1090 GDD to mature. From the same studies, average accumulated GDD during the growing season in different parts of the Great Plains ranged between 944 to 1270. Despite occasionally lowered soil temperature under long-term NT (Wang et al., 2007), available information does not support negative effects of the lowered temperature on seed germination and crop establishment on the prairies (Lafond, 1992; Lafond and Clayton, 2010).

Although detailed soil and weather temperature information on growth, development and nutrient uptake of pulse crops in the northern Great Plains is not common, lowered growth, delayed development and lower yield in response to cool temperature is likely. In the study of Malhi et al. (2007a), maximum biomass accumulation and nutrient uptake of lentil and field pea, which can be influenced by temperature, continued until the early seed-filling period. Reduced temperature in NT systems, which is likely to continue during the entire growing season (Wang et al., 2007), could restrict later growth and nutrient uptake and delay development of plants in cooler seasons.

Lowered radiation adsorption is more likely to be magnified by the accumulated crop residues in NT systems. In North Dakota, field pea yielded less in NT than in CT, and was associated with late season rainfall and accumulated GDD (Deibert and Utter, 2004). In contrast, chickpea maturity was hastened by 7 to 15 days when grown on wheat stubble compared to summer fallow in a wet-cool year in Swift Current (Gan et al., 2009b). In response to different fertility treatments in Saskatchewan, chickpea in the tilled field yielded more than it did in a no-tilled field under 0 kg N $ha^{-1}$ (Gan et al., 2009b). Since the average yield of chickpea in the 0 N treatment was smaller than in other fertilized or inoculated treatments (which all diminished the effect of tillage on yield), lowered yield in NT could be associated with soil N status.

In the northern Great Plains, maturity of lentil and chickpea is critically challenged due to their indeterminate growth habit. Hence, late season rainfall can stimulate vegetative growth, lower harvest index, delay maturity and lower quality and quantity of yield. In addition, more available

N through uptake and atmospheric fixation can intensify the effect of late rainfall on maturity and yield of indeterminate crops. In the study of Gan et al. (2009a), uninoculated chickpea with moderate amounts of N (28-84 kg N $ha^{-1}$) matured 15 days earlier than those grown under higher N fertilizer and inoculants rhizobia.

A cooler growing season during maximum growth and yield formation, which is likely in northern latitudes (Miller et al, 2002), can lower HI and yield of indeterminate crops. Post-flowering vegetative growth of lentil and chickpea favors leaf and stem expansion over yield formation (Larmure et al., 2005), and can result in delayed maturity and lowered yield (Munier-Jolain et al., 1994). Applying 150 kg N $ha^{-1}$ on two indeterminate and semi-determinate cultivars of soybean in irrigated conditions resulted in a lower yield for the indeterminate cultivar (Wallace et al., 1990). In different environments of Saskatchewan, defoliation of chickpea at vegetative growth or first flower did not noticeably affect the yield, because the crop was able to regenerate lost tissues (Li et al., 2010).

**2.6.3 Response of pulses to N**

Plant response to N fertilizer is expected to improve under NT, because more soil moisture allows greater growth, nutrient uptake and yield (Malhi et al., 2001; Kutcher and Malhi, 2010). Due to $N_2$ fixation, legume response to soil N differs from non-N fixing crops. In a study in different soil zones of western Canada, lentil yield was not increased by N fertilizer; however, lentil produced greater biomass due to the N fertilizer (Bremer et al., 1989). In contrast, a small amount of N at the beginning of growth, starter N, may increase growth and yield of pulses. In Swift Current, Saskatchewan, Gan et al. (2005) measured more yield due to the application of starter N to inoculated lentil with rhizobia. Similar results have been reported for dry pea in Alberta (McKenzie et al., 2001). In a comprehensive study in the southern part of Saskatchewan at Indian Head, Lafond et al. (2008) compared soil and crop behavior under long (25 years: LN) and short (5 years: SN) NT management. In this study, grain yield in field pea under LN and SN varied by year. While field pea yielded more in LN than in SN in 2003, it produced greater yield in SN than in LN in the next year and produced equal yield by both tillage systems in two other consecutive years. After five years of cultivation, more soil N was removed by plants from the LN than the SN field; with greater reduction in control (zero N) plots. Results of this study suggested that variation of plant growth and yield under no-till systems can be varied by temperature and rainfall. The greater N removal associated with longer can lower yield in non-legume crops, but is less likely to lower yield in legumes.

# 3.0 Common Methodologies

## 3.1 Soil N measurements

In the field studies, soil was sampled for N two weeks after seeding. Samples were taken from the middle of control plots that had not received N fertilizer prior to seeding. Soil samples were taken from depths of 0-30 and 30-60 cm and kept frozen for later analysis. In the greenhouse experiment, initial soil N content was measured by sampling from each medium, prior to filling pots. Final N content of the media was measured after harvesting of lentil at maturity by taking 20-30 g soil (media) from the middle of each pot. At the time of analysis, soils taken from two depths were bulked together, soil dried in room temperature (~25°C), and ground. Soil available N ($NO_3^-$ and $NH_4^+$) was measured by 2M KCL extraction method (Keeney and Nelson, 1982) and soil available P was measured by the modified Kelowna extraction method (Qian et al., 1994). Chemical properties of field soil and media from the greenhouse study are shown in the pertinent Chapters.

## 3.2 Plant sampling

Plant density was counted in 1-m length of three randomly selected rows in each plot, two to three weeks after germination. Biomass and N accumulation were measured at three stages full flowering (at least 80% of plants in plot flowered), full-pod (80% of plants in plot had >1 pod) and physiological maturity (80% of plants in plot turned yellow). Physiological maturity was determined as described by Tullu et al. (2001). By avoiding edge effects, the biomass samples were taken from a randomly selected 3200 $cm^2$ of each plot. Plants were cut at ground level and the shoot portions were oven-dried at 60°C for 24 hrs, weighed and ground. A sub-sample was taken for N concentration measurement by combustion method, using a Leco carbon-nitrogen determinator (LECO CNS 2000, St. Joseph, MI, USA). Total plant N content was calculated by equation 3.1 on a per plant basis, or content to a $m^2$ basis for field samples. Drying, grinding and N measurements protocol was similar for both field and greenhouse studies; time of sampling is described in details in the pertinent Chapters.

$$Plant\ N\ Content_{\ mg\ plant^{-1}} = \left[\frac{plant\ N\%}{100}\right] \times plant\ DW_{g\ plant^{-1}} \times 1000 \qquad [\mathbf{3.1}]$$

At physiological maturity, four adjacent rows in the plot, 50 cm each row, were sampled for HI. Samples were oven-dried at 60°C for 24 hrs, weighed and then thrashed for seed separation. Seed weighed, and HI was calculated by the equation 3.2. Seeds were used to determine 1000-kernel weight and then ground for seed N concentration measurement by combustion. Plots were harvested by a plot harvester, and yield was calculated based on the actual plot size, considering the sampled area.

$$HI = -\left[\frac{seed\ weight(g)}{total\ biomass\ atmaturity(g)}\right] \times 100 \qquad [\mathbf{3.2}]$$

### 3.3 $N_2$ fixation measurement

Barley was sampled with lentil for biomass at three stages of flowering, full-pod and maturity in the field studies and only at physiological maturity in the greenhouse study. Samples were dried at 60°C for 24 hr, weighed and ground. Sub-samples from lentil, pea and barley were fine ground in a ball mill for 24 hrs. A very small portion of each sample (~1 mg of lentil and ~ 3 mg of barley) was encapsulated to be analyzed for N isotopes composition on a 20-20 Mass Spectrometer interfaced with an ANCA-GSL sample converter (Europa Scientific, Crewe, UK). The proportion of atmospheric N to the total plant N *i.e. %Ndfa*, was calculated by the following equations, as described in Bremer and van Kessel, 1990 and Bremer (1991).

$$\%Ndfa = \left[ \frac{\delta^{15} N_{barley} - \delta^{15} N_{lentil}}{\delta^{15} N_{barley} - C} \right] * 1000$$

**[3.3]**

Where $\delta^{15}$N is:

$$\delta^{15}\mathrm{N} = \left[ \frac{atoms\%^{15} N_{sample} - atom\%^{15} N_{atmospher}}{atoms^{15} N_{atmospher}} \right] \quad \textbf{[3.4]}$$

The constant C, which is $^{15}$N $_{(‰)}$ of the fixing crop in an N-free medium, was assumed 0 for the lentil cultivars, as suggested in Bremer, (1991) and Bremer and van Kessel (1990). Total Fixed N was calculated by the equation 3.5 on a per plant basis, or a m$^2$ basis.

$$Total\ \ fixed\ N_{(mg\ \ plant^{-1})} = \left[ \frac{\%Ndfa}{1000} \right] \times plant\ N_{(mg\ \ plant^{-1})} \quad \textbf{[3.5]}$$

**3.3 Meteorological data**

Daily minimum, maximum and mean temperatures along with total monthly rainfall were obtained from the Environment Canada official website. Data were taken for the period of May-August for Saskatoon International airport and Indian Head. In the greenhouse study, daily temperature was recorded during the growing season. Details of weather conditions are presented for each experiment in related Chapters. Total cumulative growing-degree-days (GDD) was calculated by equation 3.6, when minimum temperature was above the base temperature ($T_b$=5°C), as explained in Miller et al. (2002) and Gan et al. (2005). The GDD accounted 0 for days with minimum temperature below the $T_b$.

$$GDD = \sum \frac{Daily\ \max T + Daily\ \min T}{2} - T_b, When\ T > 5^{\circ} C \quad \textbf{[3.6]}$$

# 4.0 Lentil Response to Varied Sources of N I. Growth, $N_2$ Fixation and Yield

## 4.1 Abstract

Lentil (*Lens culinaris* Medic.) is an indeterminate crop that continues vegetative growth after flowering. Lowered harvest index (HI) and seed yield, and delayed maturity of this $N_2$-fixing crop are likely under continuous late-season rainfall in Saskatchewan. Applying nitrogen (N) fertilizer to uninoculated lentil may inhibit atmospheric $N_2$ fixation, reduce post-flowering growth, hasten maturity, and improve HI and yield. The above ground biomass (DW) and N accumulation, $N_2$ fixation, yield, and days to maturity (DTM) of eight cultivars of lentil were studied under three fertility treatments of 50 kg N ha$^{-1}$, inoculation with granular rhizobium, and uninoculated control in Saskatchewan during 2006 and 2007.

On average, lentil produced 153, 180 and 185 g seed m$^{-2}$ in control, fertilized, and inoculated treatments, respectively. Lentil DTM was independent of the treatments, but it was hastened by 15d under a drought stress. Yield, $N_2$ fixation, DW and N content of the lentil cultivars were affected by the treatments in one location, where lentil was subjected to drought, limited soil N, and low population of indigenous rhizobia. Seed yield was associated with DW, plant N, and $N_2$ fixation, but the association of yield and HI was not strong. The cultivar CDC Plato had the best growth and yields among the cultivars and CDC Blaze the least. CDC Milestone had comparable yield, while it matured earlier than large-seeded cultivars. Results of this study do not demonstrate any advantage in applying N fertilizer to hasten maturity or increase HI and yield in lentil compared to relying on $N_2$ fixation.

## 4.2 Introduction

Saskatchewan is the greatest lentil producing province in Canada and the largest green lentil exporter in the world, yet yield and total production of this valuable crop is unpredictable in this province (Government of Saskatchewan, 2010). In addition to variable weather, the indeterminate growth habit of lentil increases the risk of delayed maturity, and lowered HI and yield. Lentil growers in the province are advised to grow early-maturing cultivars, in places that risk of frost in early fall is high (Saskatchewan Pulse Growers, 2000). The negative effects of indeterminate growth become more unpredictable with late season rainfall and excessive soil N, both of which are possible in Saskatchewan cropland.

In Melfort, SK, a cooler growing season together with greater rainfall in one out of two years increased the duration of seeding to maturity of lentil by 35 days, but reduced the amount of accumulated N in biomass and seed by 40, and 95 kg ha$^{-1}$, respectively (Malhi et al., 2007a). In a 3-year study in WI and UK, high rainfall and cool temperature extended the length from seeding to maturity in lentil (Whitehead et al., 2000). They found that lentil had substantially greater DW (up to 2000 kg ha$^{-1}$) in wet environments, but a similar yield in a drier location. In Canterbury, New Zealand, lentil yield was maximized to 3300 kg ha$^{-1}$ when rainfall dropped to 70% of the long-term average rain, but fell to about 1000 kg ha$^{-1}$ in a wet season (McKenzie and Hill, 1990).

The genetically controlled indeterminate growth habit in specific legumes can be further stimulated with more available N through atmospheric fixation or uptake from soil. Bremer et al. (1989) measured higher biomass in lentil, cultivar Laird, by applying N fertilizer; however, yield did not respond to N fertilizer, and $N_2$ fixation and HI declined. Applying 150 kg N ha$^{-1}$ to soybean (*Glycine max* L.) increased post-flowering branch growth and reduced yield of an indeterminate cultivar, whereas it slightly enhanced the yield in a semi-determinate cultivar (Wallace et al., 1990). This variation was attributed to increased lodging and disease infestation in the indeterminate soybean. When chickpea (*Cicer arietinum* L.), was grown under moderate rates of N fertilizer (28-84 kg N $ha^{-1}$), maturity of this indeterminate pulse crop was hastened (Gan et al., 2005). The earlier maturity due to the N fertilizer application was attributed to inhibited $N_2$ fixation of chickpeas by the applied N fertilizer at seeding. In Greece, applying N fertilizer to flax (*Linum usitatissimum*, L.) extended duration from flowering to maturity and improved its biomass and yield (Dordas, 2010).

Lentil is well known for the ability of $N_2$ fixation. In Saskatchewan, lentil %*Ndfa*, *i.e.* the proportion of plant N derived from $N_2$ fixation, ranged from 50% to 85%, representing 13 to 128 kg fixed $N_2$ $ha^{-1}$ (van Kessel, 1994; Bremer et al., 1988; Cowell et al., 1989). Similarly, lentil fixed 84 kg $N_2$ $ha^{-1}$ in Alberta (Rennie and Dubetz, 1986) and 62 kg $N_2$ $ha^{-1}$ in the rainfed conditions of Syria (Kurdali et al., 1997). In these studies, $N_2$ fixation remained the major source of lentil N throughout the entire growing season.

However, $N_2$ fixation of lentil, and other legume crops, can be substantially affected by environmental conditions and management. Soil N content and population of rhizobia in the rooting zone are important factors in $N_2$ fixation (Walley et al., 2001; van Kessel, and Hartley, 2000). Increasing the population of efficient rhizobia in the rooting zone via inoculation could improve $N_2$ fixation and yield of lentil (Rennie and Dubetz, 1986; Bremer et al., 1988; Badarneh and Ghawi, 1994; Gan et al., 2005), whereas applying N fertilizer at seeding resulted in contradictory effects on $N_2$ fixation and yield. In Regina, SK, lentil fixed 13.6, 16.3 and 9.6 kg $N_2$ $ha^{-1}$ and yielded 461, 490 and 512 kg seed $ha^{-1}$ in response to 10, 30 and 50 kg N fertilizer $ha^{-1}$, respectively (Cowell et al., 1989). In the semi-arid environment of Saskatchewan, response of lentil yield to 15 kg starter N $ha^{-1}$ was positive with more available water in a clay soil (Gan et al., 2005). Applying 40 kg N fertilizer $ha^{-1}$ in two different studies in Jordan (Shah et al., 2000) and Turkey (Togay et al., 2005) increased $N_2$ fixation, DW, and yield of lentil compared to 0 or 20 kg starter N $ha^{-1}$ treatments. In contrast, Bremer et al, (1989) did not find any advantages of N fertilizer application on lentil $N_2$ fixation and yield in Saskatchewan. Similarly, application of N fertilizer at seeding reduced $N_2$ fixation of chickpea and of both determinate and indeterminate cultivars of fababean (*Vicia faba* L.), which could negatively impact the yield (Doughton et al., 1993; Filek et al., 1997)

Compared to other crops, legume crops accumulate substantial amounts of N in biomass and seed; hence, yield can be restricted in low N conditions (Sinha et al., 1982). Irrigated lentil in SK accumulated 149 kg N $ha^{-1}$ compared to wheat (*Triticum aestivum* L.), which only had 98 kg N $ha^{-1}$ by maturity (van Kessel, 1994). Lentil relied on late-season $N_2$ fixation and N uptake, whereas wheat seed N was provided via N remobilization from the stem. Indeterminate crops take advantage of longer reproductive growth for greater N accumulation in seed (Dordas, 2010). Despite the great potential of lentil for $N_2$ fixation, Whitehead et al. (2000) suggested that yield and HI in lentil, especially in older cultivars, was restricted by plant N status.

Inhibiting $N_2$ fixation for maturity induction via N fertilizers in the study of Gan et al. (2009a) could reduce HI and yield. Therefore, maturity induction in lentil via lowered $N_2$ fixation would be a trade off for lowered HI and yield until a genetically determinate cultivar of lentil is developed. Applicability of this strategy is also questionable in the cropping systems of Saskatchewan, where most lentils are produced under no tillage cropping systems with high amounts of mineralizable N in soil (Sharifi et al., 2008) and continuous late season rainfall.

Under these circumstances, response of lentil $N_2$ fixation and yield to N fertilizer and rhizobia inoculants become unpredictable (Gan et al., 2005; Walley, 2001).

Response of lentil to simultaneous applications of N fertilizer and inoculant rhizobia was studied extensively in Saskatchewan, but the impact of either of these N sources on growth, yield, and especially days to maturity has not been investigated. Assuming lentil $N_2$ fixation is reduced in the presence of high available N, 50 kg $ha^{-1}$ N fertilizer should hasten maturity. When grown in an uninoculated field, lentil will have a modest $N_2$ fixation and yield due to indigenous rhizobia. This study hypothesized that applying N fertilizer will reduce $N_2$ fixation of lentil (from indigenous strains), but lentil will suffer N deficiency toward the end of season. Lentil relying on $N_2$ fixation via inoculation with a commercial strain typically fixes more N and mature later compared to lentil grown with N fertilizer. Lentil will mature earlier in fertilized plots than in inoculated plots. Significant variation is expected among environments and also cultivars belonging to various market classes.

<u>The objectives of this study were:</u>

1. *To measure the effects of soil N fertility on growth and yield of lentil*
2. *To investigate the effects of N availability on days to maturity and possible yield penalty in lentil*
3. *To estimate the effect of soil N status on lentil $N_2$ fixation*
4. *To compare lentil cultivars, for $N_2$ fixation, yield and days to maturity under varied N fertility treatments*

## 4.3 Materials and methods

### 4.3.1 Environment and cultivar selection

Field experiments were carried out in three environments (locations) of Saskatchewan during 2006 and 2007. These environments were the Goodale research farm in 2006 (GD), the Skarsgard field in 2007 (SKA) and the AAFC research farm, Indian Head, in 2007 (IH). The SKA field did not have a previous history of legume cultivation prior to the study. GD and SKA are within 6 km of each other, and located within a 30 km radius of Saskatoon, SK (52º N and 106º W); IH is located near Indian Head, SK (50° N and 103° W). Soil at GD and SKA was Dark Brown and at IH was Black. Spring soil test values for each environment are shown in Table 4.1.

**Table 4.1** Spring soil N content (kg N ha-1) at three locations of GD, SKA and IH†.

| Depth | GD | SKA | IH |
|---|---|---|---|
| 0 - 0.3 m | 15 | 13 | 19 |
| 0.3 - 0.6 m | 30 | 9 | 24 |
| Total | 45 | 22 | 43 |

†Sampling protocol and analysis method is described in Chapter Three

Eight cultivars of lentil and one each of barley (cv. CDC Dolly) and field pea (cv. Eclipse) were grown at both GD and SKA. The eight lentil cultivars belonged to different market classes: three to the large green-seeded (CDC Greenland, CDC Plato and CDC Sedley); two to the small green-seeded (CDC Milestone and CDC Viceroy); and three others to the red-cotyledon (CDC Blaze, CDC Red Rider and CDC Rouleau) market classes. At IH, two recently registered cultivars (CDC Greenland and CDC Red Rider) were omitted, and the other six cultivars were grown with Eclipse and a two-row malting barley (cv. AC Metcalfe). Barley was used as a reference crop for estimating $N_2$ fixation of lentil, as described in Chapter Three. Due to the lack of determinate cultivars in lentil, the determinate cultivar of field pea, Eclipse, was grown as a check crop for days to maturity.

### 4.3.2 Treatments and experimental designs

Lentil, field pea and barley were sown under three N fertility treatments: 1) granular rhizobium, 2) 50 kg N fertilizer $ha^{-1}$, and 3) a non-treated control. First, granular rhizobium (Becker Underwood, Saskatoon, SK) was applied at the recommended rate of 5.6 kg $ha^{-1}$ at the depth of 4 cm in the designated plots for the inoculant treatment, only. Then, the seeder was washed with 90% ethyl alcohol, and 225 g ammonium nitrate (34-0-0) $plot^{-1}$ was banded at 5 cm depth in designated plots for the fertilizer treatment. At IH, 184 g urea (46-0-0) was mid-row banded 40 cm between rows in each plot at seeding. Finally, lentil, field pea, and barley were sown at a depth of 0.03 m in all plots. Plot size was 3.2× 4.9 m (15.7 $m^{-2}$) with 0.20 m of plant row spacing at GD and SKA and 1.6×10.7 m (17.1 $m^{-2}$) with 0.20 m row spacing at IH. Seeding dates were May 2, 2006 at GD, May 15, 2007 at SKA and May 5, 2007 at IH.

A RCBD design with a split-plot arrangement of the N fertility treatments and cultivars was used at GD and SKA. Three N fertility treatments were randomized in the main plots and cultivars were in the sub-plots. The position of barley plots relative to lentil and field pea plots differed slightly at GD and SKA. At GD, barley was randomized among the lentil and field pea within each main plot, but at SKA, barley was sown alongside each main plot. Therefore, each lentil (and pea) plot at SKA had an adjacent barley plot. Both experiments at GD and SKA had treatments repeated in four blocks. At IH, a RCBD design with factorial combinations of the N fertility treatments and cultivars (including barley and field pea) was assigned in three replications. Hence, each N fertility treatment had one barley plot and one field pea plot per replication. Plot maintenance, sampling, and analysis of samples for total N and fixed $N_2$ were similar among the environments, as described in Chapter Three.

### 4.3.3 Data analysis

Due to different soil and weather conditions, varied experimental designs, and different cultivar and replication numbers among the environments, data were analyzed for each environment separately. For DW, N and $N_2$ fixation, collected data were analyzed for each growth stage-environment, separately. Data were analyzed as a nested design in Proc Mixed of SAS, version 9.2 (SAS Institute, Cary, NC) with the main plots as N fertility treatments (inoculant, N fertilizer, control), and the sub-plots as cultivars. Presented results in this chapter are all based on separate analysis of variance for each environment; however, average data over the environments was occasionally used for interpreting the results.

## 4.4 Results

### 4.4.1 Weather conditions

Average monthly mean temperature and total rainfall during lentil growth varied among the environments. In general, GD had a warmer growing season and better rainfall distribution for lentil yield (sufficient rain during growth and a mild drought at maturity) than the other two environments. Specifically, the mean temperature during August, when lentil was maturing, was ~2.5°C warmer at GD (2006) than at the other two locations-years. During the two years of study, total rainfall from May to August was equal among the three environments and lentil received a total of 210, 216 and 206 mm rain at GD, SKA and IH, respectively; however, the rainfall distribution varied substantially among the environments. At GD, sufficient rainfall throughout the season was followed by a mild drought. At SKA, low rainfall in July (22 mm) and August (17.5 mm) resulted in a severe mid-season drought. At IH, late-season rainfall continued and lentil received 52, and 63 mm rain in July and August, respectively (Table 4.2).

**Table 4.2** Average monthly temperatures and total monthly rainfall from Saskatoon international airport (for GD and SKA) and Indian Head, CDA station (for IH). Monthly temperatures are based on the daily mean, minimum and maximum temperatures.

| | | **Temperature** | | | **Rainfall** | **Long-term means†** | |
|---|---|---|---|---|---|---|---|
| **Environment** | **Month** | **Mean** °C | **Max** °C | **Min** °C | **monthly** mm | **Temperature** °C | **Rainfall** Mm |
| **GD (2006)** | May | 12 | 18 | 5 | 39 | 12 | 47 |
| | Jun | 16 | 22 | 10 | 108 | 16 | 61 |
| | Jul | 20 | 27 | 13 | 32 | 18 | 60 |
| | Aug | 18 | 26 | 10 | 30 | 17 | 38 |
| **SKA (2007)** | May | 11 | 18 | 5 | 46 | 12 | 47 |
| | Jun | 15 | 22 | 8 | 131 | 16 | 61 |
| | Jul | 21 | 28 | 14 | 22 | 18 | 60 |
| | Aug | 16 | 23 | 9 | 18 | 17 | 38 |
| **IH (2007)** | May | 10 | 16 | 3 | 46 | 11 | 83 |
| | Jun | 15 | 22 | 8 | 46 | 16 | 79 |
| | Jul | 20 | 27 | 13 | 51 | 18 | 67 |
| | Aug | 16 | 22 | 9 | 63 | 18 | 53 |

†Averages for the period of 1970-2000

### 4.4.2 Analysis of variances

Separate analysis of variance for each environment showed that days to maturity (DTM) of lentil was independent of the fertility treatments across all environments (Table 4.3, 4.4). Seed yield, seed N concentration, and final plant N content, *%Ndfa* and fixed $N_2$ were affected by the fertility treatments at SKA only (Tables 4.3, 4.4). At this location, initial soil N was less than in GD and IH (Table 4.1) and lentil experienced a mid-season severe drought stress. At IH, only DW was affected by the fertility treatments, and at GD, lentil did not respond to the fertility treatments.

Cultivars varied for DTM, yield, and seed N concentration in all environments (Table 4.3). Harvest index differed among the cultivars at GD and IH, but not at SKA. Noticeable leaf loss together with lowered yield under dry conditions of SKA, may be associated with the similar HI among the eight cultivars of lentil.

Interaction of the cultivar and N fertility treatments was significant for yield at IH and for DTM at SKA. These interactions were more likely associated with varied $N_2$ fixation and biomass accumulation, varied plant density, and likely related to plot position and water availability. At IH, variation in stand counts among the lentil cultivars was observed (Fig. 4.4).

**Table 4.3** Effects of the N fertility treatments, cultivars and their interaction on lentil growth, N and yield in three environments. Results of separate analysis of variance for each environment.

| | | **Yield** (g m$^{-2}$) | | | **HI** (%) | | | **Seed N** (%) | | | **DTM** (d) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Effect** | DF | GD | SKA | IH | GD | SKA | IH | GD | SKA | IH | GD | SKA | IH |
| **N treatment (T)** | 2 | ns | * | ns | ns | ns | ns | ns | ** | ns | ns | ns | ns |
| **Cultivar ( C)** | 7 | ** | ** | ** | * | ** | * | ** | ns | ** | ** | ** | ** |
| **C × T** | 14 | ns | Ns | * | ns | ns | ns | ns | ns | ns | ns | * | ns |
| Plant DW and N at physiological maturity | | | | | | | | | | | | | |
| | | **DW** (g m$^{-2}$) | | | **Total N** (g m$^{-2}$) | | | ***%Ndfa*** | | | **Fixed N** (g m$^{-2}$) | | |
| **Effect** | DF | GD | SKA | IH | GD | SKA | IH | GD | SKA | IH | GD | SKA | IH |
| **N treatment (T)** | 2 | ns | ns | * | ns | * | ns | ns | ** | ns | ns | ** | ns |
| **Cultivar ( C)** | 7 | * | ns | ns | * | * | ns | ns | ns | ns | ns | ** | ns |
| **C × T** | 14 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

*, ** and ns indicate significant ($P<0.05$), highly significant ($P<0.01$) and not significant differences among N fertility treatments, cultivars and their interactions with environments

### 4.4.3 Days to maturity, yield, DW and N

In general, the lentil cultivars matured 84, 102 and 101 days after seeding (DAS) at SKA, GD and IH, respectively. The earlier maturity of lentil at SKA was related to drought stress and N deficiency. At both GD and SKA, lentil matured due to drought, but at IH desiccant was applied prior to harvesting. Lentil DTM, was independent of the fertility treatments, and lentil matured within 3 days among the fertility treatments (Table 4.4). Any possible effect of fertility treatments on lentil DTM would have occurred at SKA, where lentil growth and yield differed by the fertility treatments. However, severe drought in July and in August forced lentil to dry at this location.

Average seed yield at GD and IH was identical (218 g $m^{-2}$), and greater than at SKA (173 g $m^{-2}$). The similar yield of lentil at GD and IH, despite higher yield potential in the black soil at IH, is also explained by the absence of two high-yielding cultivars, CDC Greenland and CDC Red Rider, at IH. Effects of the N fertility treatments on seed yield was observed at SKA only, where lentil produced 30g greater seed $m^{-2}$ in both fertilized and inoculated plots than in the control. Average yield of lentil in the treated plots (inoculant and N fertilizer) at GD and IH was 26 and 23 g $m^{-2}$ greater than in the control plots, respectively; however, the differences were not significant (Table 4.4).

**Table 4.4** Average yield, growth and N of lentil grown under three N fertility treatments of control, N fertilizer and inoculant in GD, SKA and IH (means of cultivars and replications).

| Fertility Treatments | **Seed yield** (g $m^{-2}$) | | | **HI** (%) | | | **Seed N** (%) | | | **DTM** (d) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GD | SKA | IH | GD | SKA | IH | GD | SKA | IH | GD | SKA | IH |
| Control | $205^{a}$ | $153^{b}$ | $211^{a}$ | $45^{a}$ | $40^{a}$ | $50^{a}$ | $4.0^{a}$ | $3.7^{b}$ | $3.9^{a}$ | $101^{a}$ | $84^{a}$ | $101^{a}$ |
| N Fertilizer | $221^{a}$ | $180^{a}$ | $234^{a}$ | $43^{a}$ | $43^{a}$ | $52^{a}$ | $3.9^{a}$ | $3.5^{c}$ | $3.9^{a}$ | $101^{a}$ | $86^{a}$ | $101^{a}$ |
| Inoculant | $228^{a}$ | $185^{a}$ | $208^{a}$ | $43^{a}$ | $44^{a}$ | $52^{a}$ | $4.0^{a}$ | $3.9^{a}$ | $3.8^{a}$ | $104^{a}$ | $83^{a}$ | $101^{a}$ |

Plant DW and N at physiological maturity

| Fertility Treatments | **DW** (g $m^{-2}$) | | | **Total N** (g $m^{-2}$) | | | ***Ndfa*** (%) | | | **Fixed N** (g $m^{-2}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GD | SKA | IH | GD | SKA | IH | GD | SKA | IH | GD | SKA | IH |
| Control | $673^{a}$ | $390^{b}$ | $437^{b}$ | $13.2^{a}$ | $8.5^{b}$ | $9.1^{a}$ | $59^{ab}$ | $63^{a}$ | $64^{b}$ | $7.3^{a}$ | $5.3^{a}$ | $6.0^{b}$ |
| N Fertilizer | $688^{a}$ | $427^{a}$ | $464^{ab}$ | $11.5^{a}$ | $9.2^{ab}$ | $8.8^{a}$ | $69^{ab}$ | $24^{c}$ | $64^{b}$ | $7.8^{a}$ | $2.2^{b}$ | $7.3^{ab}$ |
| Inoculant | $691^{a}$ | $430^{a}$ | $480^{a}$ | $11.8^{a}$ | $9.6^{a}$ | $9.1^{a}$ | $76^{a}$ | $60^{ab}$ | $84^{a}$ | $8.9^{a}$ | $5.8^{a}$ | $7.6^{ab}$ |

Means followed by same letter within columns indicate non-significant difference among the N fertility treatments or cultivars ($P<0.05$)

Harvest index at IH (52%) noticeably exceeded the HI in both high-yielding GD (44%) and low-yielding SKA (42%) locations (Table 4.4). The unexpected high HI of 52% at IH may be related to lost leaves, which were not included in the total biomass value used to calculate HI. Biomass loss occurred toward lentil maturity at IH, with the greatest loss in non-treated control (Fig. 4.1). Lentil HI did not respond to the N fertility treatments in any environment (Table 4.4).

At maturity, $N_2$ fixation varied among the three environments: mean fixed N were 8.0, 4.4 and 7.0 g N $m^{-2}$at GD, SK and IH, respectively (Table 4.4). Total fixed N was calculated by %*Ndfa* × DW × Plant N concentration. In response to the fertility treatments, total fixed $N_2$ of lentil was different at SKA only, but in the other two locations, total fixed N was equal among the fertility treatments (Table 4.4). The fertilizer and control treatments were not inoculated; thus, any $N_2$ fixation under these treatments depended on the efficiency of indigenous soil rhizobia for fixing $N_2$. Variable responses of $N_2$ fixation to the fertility treatments in different environments were explained by soil N content, soil cultivation and inoculation histories, and weather conditions. Unlike total fixed N, %*Ndfa* was sensitive to the fertility treatments. For example, total fixed $N_2$ of lentil at IH did not differ by the fertility treatments; however, the inoculant treatment had greater %*Ndfa* than both fertilizer and control treatments (Table 4.4). The smaller amount of fixed $N_2$ (g $m^{-2}$) in the fertilized plots was associated with a lower lentil %*Ndfa* in this treatment (Fig 4.1).

Plant DW and plant N content varied among the environments. By maturity, lentil cultivars accumulated 684, 416 and 460 g DW $m^{-2}$ at GD, SKA and IH, respectively. Despite more late-season rain at IH than at SKA (Table 4.2), final N content of lentil was equal at both locations, likely due to noticeable biomass (leaf) loss at IH during podding to maturity (Fig 4.1). In response to the fertility treatments, lentil by maturity produced greater DW in the treated plots than in the control at both SKA and IH (Table 4.3). This variation was related to less available N in the control, because plant N concentration in the control treatment was also lower (Table 4.4).

Seed N concentration was affected by water and N availability among the environments. Seed N concentration at SKA (3.7%) was less than at GD (4.0%) and IH (3.9%). The synchronized low seed yield and low seed N concentration at SKA showed that lentil experienced N deficiency, due to the severe drought that reduced growth and N uptake and hastened maturity of lentil by ~20 days. The fertility treatments resulted in varied seed N concentration of lentil at SKA only. At this location, seed N concentration was maximized in the inoculant treatment (3.9%), followed by the non-treated control (3.7%) and then in the N fertilizer treatment (3.5%). Varied seed N concentration among the N fertility treatments at SKA, despite similar yield of lentil in inoculant (185 g $m^{-2}$) and fertilizer (180 g $m^{-2}$) treatments, showed the effect of N source on seed N. At this location, continuous late season $N_2$ fixation in the inoculated treatment could increase the seed N concentration, but early N uptake from the N fertilizer source was tied up in the stems (more details in Chapter 5). Seed N concentration in field crops is associated with plant N status; however, N concentration may be altered by yield quantity and ability of cultivars for N remobilization.

**4.4.4 Vegetative growth and N accumulation**

At flowering, lentil DW was equal among the three environments, but plant N concentration, total plant N content, %*Ndfa,* and total fixed $N_2$ at SKA was lower than in the other two locations. Concentration of plant N at flowering was 4% in lentil grown at GD and IH, and 3% in lentil grown at SKA (data not presented). Despite similar rainfall across environments until flowering (Table 4.2), substantially low N concentration of lentil at SKA suggested that lentil there was subjected to N deficiency from the beginning of the season. At this location, the control treatment resulted in a lower plant N than the other two treatments, because of inefficient $N_2$ fixation of lentil in symbiosis with indigenous rhizobia (Fig 4.1). Also, inoculated lentil at SKA accumulated less N by flowering than inoculated lentil grown at the other locations by flowering, indicating that initial soil N, which is necessary for plant growth prior to nodulations, was inadequate at SKA. Soil available N at seeding was less in SKA than in GD and IH (Table 4.1).

Fertilizer treatment inhibited $N_2$ fixation of lentil by flowering at both SKA and IH. By flowering, lentil %*Ndfa* in the control, N fertilizer, and inoculant treatment was respectively 7, 2, and 14% (at SKA) and 61, 0, and 67% (at IH). At GD, early season %*Ndfa* seemed to be limited by insufficient initial soil N rather than excessive N, because the %*Ndfa* was less in the control (17%) than in the fertilizer treatment (34%). Although total soil N at seeding was similar at both GD and IH, most of the soil N at GD was accumulated at greater depth (Table 4.1). Unexpectedly, lentil *%Ndfa* at IH decreased from full-pod to maturity by 19, 21 and 9% in the control, fertilizer, and inoculant treatments, respectively (data not presented). The lowered %*Ndfa* at IH could be associated with either lost leaves which enriched the atmospheric $N_2$ value (see also the DW loss in Fig 4.1), or, less likely due to an increased soil N uptake which diluted plant N originating from the atmosphere.

While sufficient N across three fertility treatments at GD resulted in similar DW, lentil DW in the control treatment in the other environments was restricted by N shortage (Fig 4.1). For example, at HI, lentil lost more biomass during to maturity in the control (N-deficient treatment) than in fertilizer and inoculated treatments. Likewise, lack of sufficient N in the control treatment lowered DW accumulation of lentil at SKA (Fig 4.1).

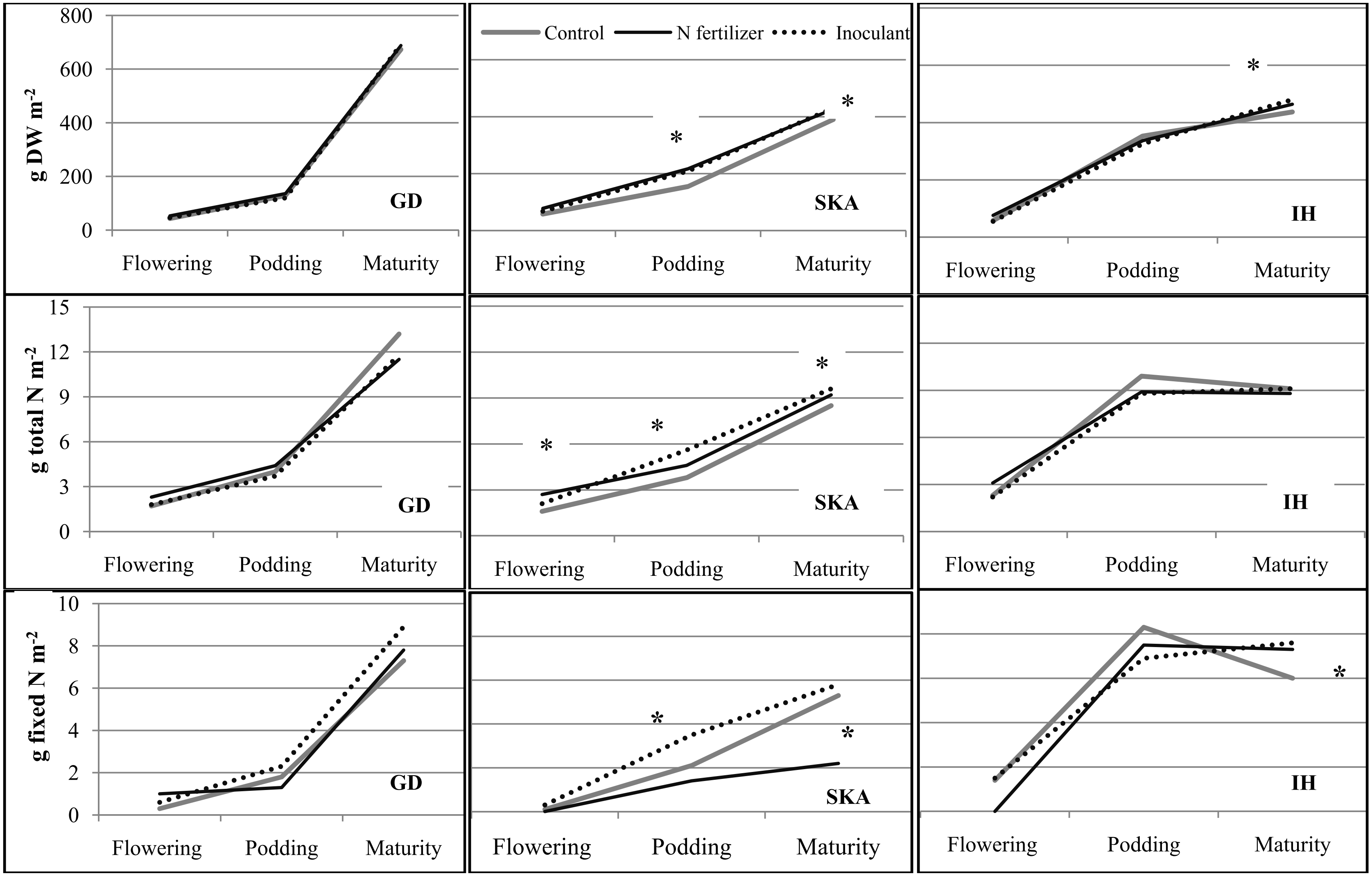


**Figure 4.1** Average DW (upper panel) total plant N (middle panel) and fixed $N_2$ (lower panel) in response to the fertility treatments at three stages of flowering, podding and maturity at Goodale (GD), Skarsgard (SKA) and Indian Head (IH).

* Indicates significant differences of means at each stage, otherwise means are not different at P <0.05

### 4.4.5 Cultivar variation

Cultivars were significantly different for days to maturity. Across all three environments, small seeded cultivars matured before the large-seeded cultivars. At GD and SKA, CDC Blaze had the earliest, and CDC Greenland had the latest days to maturity; other cultivars were in between. In IH, where cultivars CDC Greenland and CDC Red Rider were absent, CDC Sedley and CDC Plato matured later than other cultivars (Table 4.5). Days to maturity of the lentil cultivars were occasionally independent of the lentil market class. For example, DTM of CDC Rouleau (red-cotyledon, early maturing cultivar) at GD and IH was not different from DTM of the late maturing cultivars CDC Plato and CDC Sedley. At IH, where DTM of CDC Blaze was missing, cultivar DTM was divided into three groups representing large green-seeded cultivars (107d), red-cotyledon cultivars (100d), and small green-seeded cultivars (96d).

**Table 4.5** Average days to maturity (DTM), yield, HI and seed N concentration of different cultivars of lentil at Goodale (GD), Skarsgard (SKA) and Indian Head (IH). Means are averaged over three N fertility treatments and four replications.

| Cultivars | **DTM** (d) | | | **Yield** (g $m^{-2}$) | | | **HI** (%) | | | **Seed N** (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GD | SKA | IH | GD | SKA | IH | GD | SKA | IH | GD | SKA | IH |
| **CDC Blaze** | $94^{e}$ | $82^{c}$ | § | $188^{d}$ | $130^{d}$ | $131^{c}$ | $47^{a}$ | $35^{b}$ | $56^{a}$ | $4.1^{a}$ | $4.0^{a}$ | $3.9^{a}$ |
| **CDC Rouleau** | $101^{bcd}$ | $83^{bc}$ | $100^{b}$ | $205^{cd}$ | $174^{bc}$ | $194^{b}$ | $41^{b}$ | $45^{a}$ | $54^{a}$ | $3.8^{de}$ | $3.6^{cd}$ | $3.8^{a}$ |
| **CDC Red Rider** | $100^{cd}$ | $86^{ab}$ | --- | $231^{ab}$ | $195^{a}$ | --- | $45^{ab}$ | $45^{a}$ | --- | $3.9^{bc}$ | $3.8^{b}$ | --- |
| **CDC Milestone** | $96^{de}$ | $82^{bc}$ | $96^{c}$ | $214^{bc}$ | $170^{bc}$ | $202^{b}$ | $46^{a}$ | $41^{a}$ | $55^{a}$ | $3.9^{cde}$ | $3.4^{e}$ | $3.8^{a}$ |
| **CDC Viceroy** | $104^{abc}$ | $82^{c}$ | $96^{c}$ | $243^{a}$ | $168^{c}$ | $263^{a}$ | $44^{ab}$ | $43^{a}$ | $50^{b}$ | $4.2^{a}$ | $3.8^{b}$ | $4.0^{a}$ |
| **CDC Greenland** | $109^{a}$ | $89^{a}$ | --- | $250^{a}$ | $183^{abc}$ | --- | $40^{b}$ | $44^{a}$ | --- | $4.0^{b}$ | $3.7^{bc}$ | --- |
| **CDC Plato** | $108^{a}$ | $87^{a}$ | $107^{a}$ | $233^{ab}$ | $186^{ab}$ | $282^{a}$ | $45^{ab}$ | $42^{a}$ | $49^{b}$ | $3.8^{e}$ | $3.6^{cd}$ | $3.7^{a}$ |
| **CDC Sedley** | $106^{ab}$ | $87^{a}$ | $107^{a}$ | $180^{d}$ | $175^{bc}$ | $235^{ab}$ | $42^{ab}$ | $44^{a}$ | $45^{c}$ | $3.9^{bcd}$ | $3.7^{bc}$ | $4.0^{a}$ |
| **Eclipse (pea)** | $101^{bcd}$ | $80^{c}$ | $94^{d}$ | --- | --- | --- | --- | --- | --- | --- | --- | --- |

Means followed by same letter within columns indicate non-significant difference among the N fertility treatments in different growth stages or cultivars (P<0.05)
--- Data were not collected or cultivar was absent
§ Missing value

The determinate check field pea, cv. Eclipse, had similar or earlier DTM than lentil cultivars at SKA and IH, but at GD matured later than CDC Blaze and CDC Milestone. Eclipse in the inoculant treatment matured earlier than in the fertilizer treatment at SKA (Fig 4.2), which implied that N fixation in the field pea was inefficient under drought at SKA. Interaction of cultivar × N fertility treatment for DTM occurred at SKA. Here, maturity of CDC Blaze, CDC Rouleau and CDC Viceroy in the fertilizer and control treatments was delayed compared to the inoculant treatment. The other cultivars had almost similar DTM in all three N fertility treatments (Fig. 4.2).

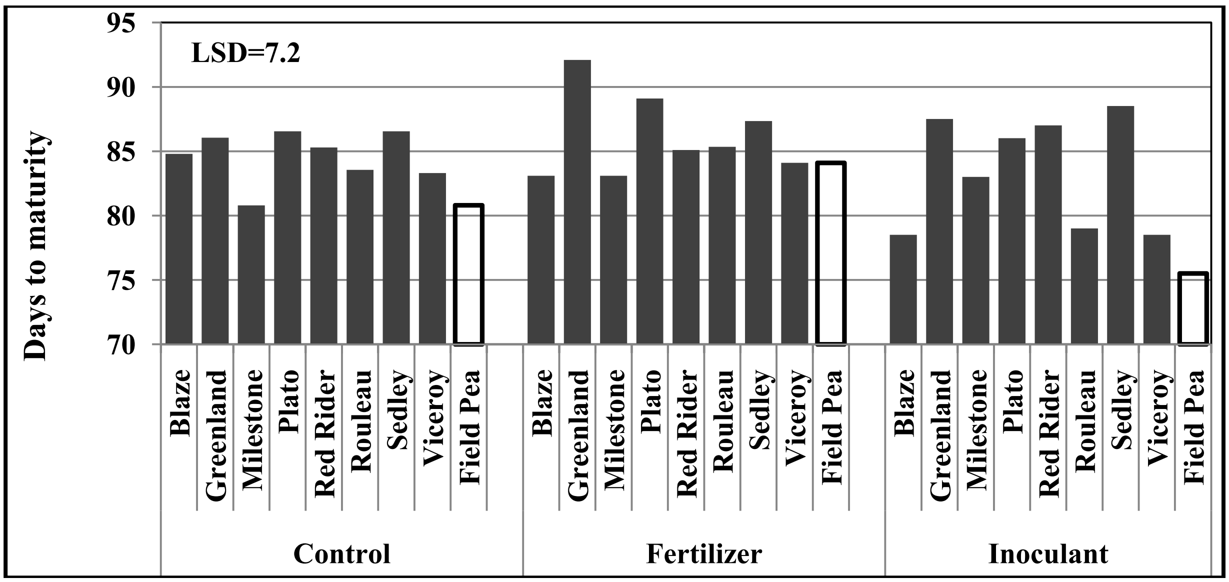


**Figure 4.2** Days to maturity of eight lentil cultivars and pea grown under three N fertility treatments at SKA (means of four replications). Prefix CDC was omitted from the cultivars names.

The late-maturing, large-seeded cultivars produced more yield than the early-maturing, small-seeded cultivars. When data were averaged over the environments, N fertility treatments, and replications, CDC Plato produced the greatest and CDC Blaze had the smallest yield among the cultivars (Table 4.5). Yield of the large-seeded CDC Sedley was different in three environments. At IH, yield of CDC Sedley did not differ from the maximum yield (cv. Plato and Viceroy), but at GD and SKA it did not produce as much yield as other cultivars (Table 4.5). The smaller yield of CDC Sedley than other large-seeded cultivars at GD and SKA may be associated with its lowered $N_2$ fixation.

Unlike CDC Viceroy, which had reduced yield under drought stress at SKA, CDC Red Rider and CDC Greenland were better adapted to varied weather conditions and produced greater yield than most of the other cultivars in both suitable (GD) or stressed (SKA) environments. These cultivars were absent at IH. Slightly lower yield of inoculated lentil than fertilized lentil, and less variation among the cultivars for yield in the inoculant treatment than in other treatments were observed at IH (Table 4.4, Fig. 4.3). Interaction of cultivars and N fertility treatments for seed yield was observed at IH, where CDC Rouleau had less yield in the fertilized plots than in the non-fertilized plots (control and inoculants). This lower yield was independent of plant density, since CDC Rouleau had 76, 98 and 78 plant $m^{-2}$ in the inoculant, fertilizer, and control treatments, respectively (Fig 4.4).

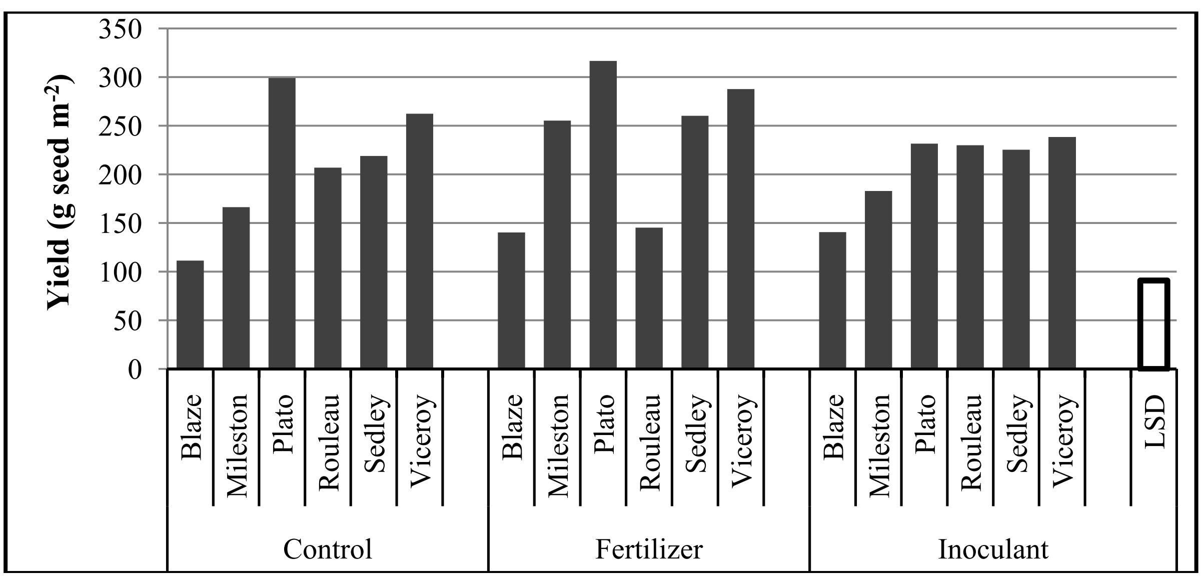


**Figure 4.3** Average seed yield of lentil cultivars under three N fertility treatments of control, fertilizer and inoculant at IH, means of three replications. Prefix CDC was omitted from the cultivars names.

Harvest index differed among the lentil cultivars, with CDC Blaze and CDC Milestone having the greatest HI. Surprisingly, CDC Blaze had the smallest HI amongst the cultivars at SKA, where seed yield was strongly suppressed by drought and N deficiency. CDC Sedley always had one of the smallest HI amongst the cultivars. The high-yielding cultivars CDC Greenland and CDC Plato had lower HI values than some of the small-seeded cultivars in all environments. Although the HI of low-biomass cultivars may exceed the HI of large-biomass cultivars under suitable conditions (like GD and IH), HI may still be remarkably reduced under drought and low N conditions as shown by CDC Blaze at SKA (Table 4.6). Varied HI-yield associations among the environments suggested that HI may have less effect on yield under cool growing season, when seed N accumulation rate is lowered by insufficient heat (see HI of CDC Sedley and CDC Plato at IH in table 4.5).

As expected, cultivar DW values were different, and large-seeded cultivars had greater DW than small-seeded cultivars. Averaged over the environments and fertility treatments, CDC Plato had the greatest (543 g $m^{-2}$) and CDC Blaze the smallest (481 g $m^{-2}$) DW among the cultivars. Differences of DW among the cultivars were not always significant (Table 4.6). The greater DW of CDC Plato compared with other cultivars in the drought conditions of SKA was associated with its early season DW accumulation. In this environment, CDC Plato accumulated significantly greater DW than most other cultivars by full-pod (data not presented).

Total plant N content at maturity was not different among the cultivars, except for CDC Blaze at GD, and CDC Sedley at SKA, both of which had a lower total N content than the other cultivars. The synchronized low DW and low N% of CDC Blaze and CDC Sedley at SKA reflected their low $N_2$ fixation (Table 4.6), which also lowered seed yield.

**Table 4.6** Average DW, N content, %Ndfa and fixed N2 by lentil cultivars at Goodale (GD), Skarsgard (SKA) and Indian Head (IH). Means are averaged over three N fertility treatments and four replications).

| | **DW** (g m$^{-2}$) | | | **N content** (g m$^{-2}$) | | | ***Ndfa*** (%) | | | **Fixed N** (g m$^{-2}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cultivar** | GD | SKA | IH | GD | SKA | IH | GD | SKA | IH | GD | SKA | IH |
| **CDC Blaze** | 610$^{c}$ | 388$^{b}$ | 446$^{ab}$ | 9.2$^{c}$ | 9.1$^{abc}$ | 8.6$^{a}$ | 77$^{a}$ | 40$^{c}$ | 76$^{a}$ | 7.0$^{b}$ | 3.6$^{bc}$ | 6.7$^{a}$ |
| **CDC Rouleau** | 686$^{abc}$ | 400$^{ab}$ | 429$^{b}$ | 13.1$^{ab}$ | 8.9$^{abc}$ | 9.4$^{a}$ | 61$^{b}$ | 53$^{abc}$ | 66$^{a}$ | 7.8$^{ab}$ | 4.6$^{ab}$ | 7.6$^{a}$ |
| **CDC Red Rider** | 652$^{bc}$ | 436$^{ab}$ | --- | 11.7$^{bc}$ | 9.9$^{ab}$ | --- | 67$^{ab}$ | 51$^{abc}$ | --- | 7.5$^{ab}$ | 5.0$^{ab}$ | --- |
| **CDC Milestone** | 652$^{bc}$ | 421$^{ab}$ | 470$^{ab}$ | 12.9$^{ab}$ | 9.9$^{ab}$ | 8.9$^{a}$ | 65$^{ab}$ | 59$^{ab}$ | 74$^{a}$ | 8.3$^{ab}$ | 5.7$^{a}$ | 7.6$^{a}$ |
| **CDC Viceroy** | 753$^{ab}$ | 407$^{ab}$ | 437$^{b}$ | 12.4$^{ab}$ | 8.5$^{bc}$ | 8.3$^{a}$ | 71$^{ab}$ | 44$^{abc}$ | 64$^{a}$ | 8.4$^{ab}$ | 3.6$^{bc}$ | 6.0$^{a}$ |
| **CDC Greenland** | 791$^{a}$ | 423$^{ab}$ | --- | 15.1$^{a}$ | 8.6$^{bc}$ | --- | 65$^{b}$ | 43$^{abc}$ | --- | 9.5$^{a}$ | 3.7$^{bc}$ | --- |
| **CDC Sedley** | 654$^{bc}$ | 402$^{ab}$ | 475$^{ab}$ | 11.1$^{bc}$ | 7.7$^{c}$ | 8.4$^{a}$ | 72$^{ab}$ | 43$^{bc}$ | 82$^{a}$ | 8.0$^{ab}$ | 3.3$^{c}$ | 7.3$^{a}$ |
| **CDC Viceroy** | 753$^{ab}$ | 407$^{ab}$ | 437$^{b}$ | 12.4$^{ab}$ | 8.5$^{bc}$ | 8.3$^{a}$ | 71$^{ab}$ | 44$^{abc}$ | 64$^{a}$ | 8.4$^{ab}$ | 3.6$^{bc}$ | 6.0$^{a}$ |

Means followed by same letter within columns indicate non-significant difference among the N fertility treatments in different growth stages or cultivars ($P<0.05$)
--- Cultivars were absent

At maturity, %*Ndfa* was different among the lentil cultivars at GD and SKA, but not at IH. At GD, CDC Blaze and CDC Greenland had the greatest and the smallest %*Ndfa* amongst the cultivars, respectively (Table 4.6). In contrast, CDC Blaze had the smallest %*Ndfa* among the cultivars at SKA. This variation among cultivars and environments suggested that %*Ndfa* was independent of DW and N content, but may be associated with plant $N_2$ fixation. At GD, greater N content of CDC Plato diluted the proportion of fixed $N_2$ (%*Ndfa*), but at SKA, the ability of cultivars to maintain DW and N content resulted in greater %*Ndfa* under the drought stress. When the total amount of fixed $N_2$ was considered, the lowest $N_2$ fixation belonged to CDC Blaze at GD and CDC Sedley at SKA (Table 4.6).

Seed N concentration did not vary by cultivar in the wet-cool conditions of IH, but differed at two other locations. At both GD and SKA, CDC Blaze, which produced the smallest seed yield, had the greatest seed N concentration among the cultivars. Conversely, CDC Milestone had simultaneously low yield and low seed N concentration at SKA. Stand count, which was always lower than the targeted density of 100 plants m$^{-2}$, varied among the environments. Average stand counts at GD, SKA and IH were 93, 82 and 82 plants m$^{-2}$, respectively (Table 4.4). At IH, stand counts varied among plots having different cultivars, possibly because of seeds coming from different sources (Fig 4.1). Varied performance of lentil cultivars appeared independent of plant density. For example, CDC Plato had one of the lowest stand counts at IH (Fig. 4.4), but produced the greatest yield among the cultivars in this environment (Table 4.5, Fig 4.3).

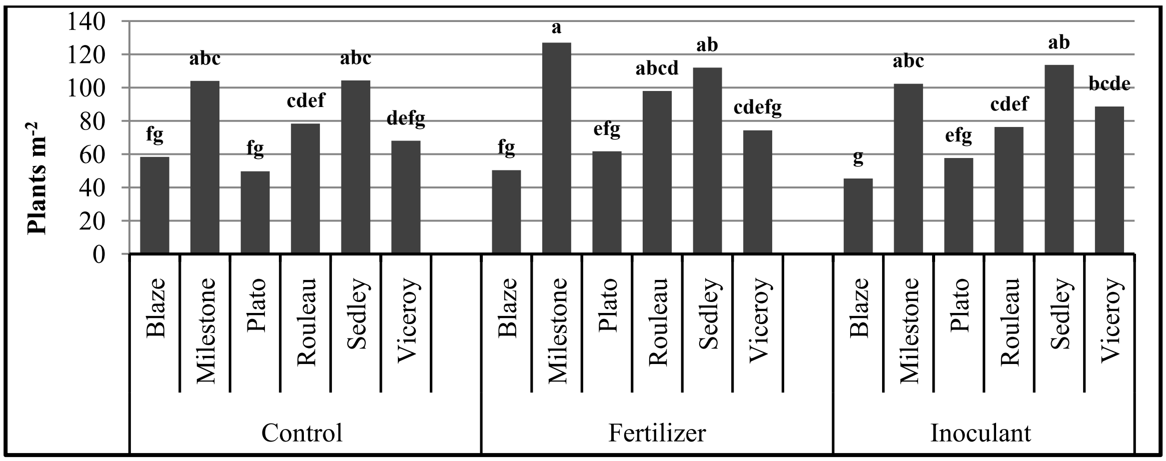


Figure 4.4 Average stand counts of lentil in plots that had different N fertility treatments and cultivars at IH (means of three replications). Similar letters indicate non-significant differences among lentil cultivars within each treatment (P<0.05).

## 4.5 Discussion

### 4.5.1 Maturity and nitrogen

Applying 50 kg $ha^{-1}$ N fertilizer rather than the inoculated treatment in lentil did not hasten maturity nor improve yield. Lentil matured regardless of the three fertility treatments under both dry and wet conditions. This result was also in agreement with the findings on lentil by Gan et al. (2005), who measured only 0.5 to 1.3d earlier maturity in lentil with N fertilizer than in inoculated lentil across six environments of Saskatchewan. The fertility treatment had a similar effect on lentil maturity. The lack of difference in lentil maturity in the current study was also seen with similar final plant N content (Table 4.4). For example, lentil %*Ndfa*, the best indicator of $N_2$ fixation efficiency, was restricted under both fertilized and control plots in the cool-wet environment of IH, but total plant N content remained independent of the %*Ndfa* (Table 4.4). On one occasion at SKA, where final N content of lentil differed by the fertility treatments, severe drought stress hastened lentil maturity.

Stimulated early growth of lentil N across the fertility treatments at IH did not last long enough to alter plant maturity. The greater lentil DW and N content at IH compared to the other locations at both flowering and podding disappeared by maturity (Fig 4.1). In contrast to these results, chickpea grown with moderate rates of 28 to 84 kg N fertilizer $ha^{-1}$, matured earlier than chickpea grown without the N-fertilizer (Gan et al., 2009a), possibly due to inhibited $N_2$ fixation and lowered N plant content by the N fertilizer. My study showed that 50 kg N $ha^{-1}$ occasionally inhibited lentil $N_2$ fixation, but did not affect total final plant N content in any environment. Restricted fixed $N_2$ (g $m^{-2}$) in the fertilized plots at SKA was compensated by improved N uptake from soil and fertilizer, and both inoculated and fertilized treatments had equal N content by maturity (Table 4.4). Lower $N_2$ fixation of legume crops in the presence of sufficient N (Bremer et al., 1989; Walley, 2001; Doughton et al., 1993; Filek et al., 1997) can be compensated via more N uptake in places like Saskatchewan, where the soil N mineralization rate is high (Sharifi et al., 2008; Gan et al., 2005; Walley, 2001).

Plant maturity is possibly accelerated under N deficient circumstances, when N remobilization from leaves triggers leaf senescence and induces maturity (Schiltz et al., 2005). In indeterminate soybean, days to maturity was associated with N remobilization rate, which occurred in N-deficient treatments (Munier-Jolain et al., 1996). Although N remobilization of lentil was not measured in my study, the almost 20d earlier maturity of lentil in SKA than in other locations may be associated with greater N remobilization from leaves under drought pressure (see Chapter 5, and Kurdali et al., 1997 and van Kessel, 1994). With late-season cool-wet conditions, like IH, both N uptake and N dedication to seeds may be different from drier conditions (Malhi et al., 2007a; Larmure et la., 2005).

Average N accumulation of lentil across environments was 102 kg N $ha^{-1}$, slightly greater than the 98 kg N $ha^{-1}$ in the irrigated experiment by van Kessel, (1994); however, lentil matured 19 days earlier than in the in the van Kessel study. In a winter seeding date in Syria, a late maturing cultivar (Kurdi) accumulated 32 kg less N $ha^{-1}$, but matured almost 10 days later than four other lentil cultivars (Kurdali, 1997). Considering outcomes of other lentil researches together with the varied performance of lentil cultivars in my study, results suggest that weather had a greater effect on maturity of lentil than plant N status (Gan et al., 2005; Whitehead et al., 2000; McKenzie and Hill, 1995).

Effects of inoculation and N fertilizer treatments varied by soil cultivation history, soil initial N, and weather conditions. The greatest benefits of the inoculant treatment was observed at IH, where lentil had access to more water, and at SKA, where lentil growth was restricted by drought and N deficiency. Varied response of lentil inoculated with efficient strains of rhizobia has been emphasized previously (Rennie and Dubetz, 1986; Gan, et al., 2005). Similarly, when the average yield of lentil and pea together ranged 488 to 3314 kg $ha^{-1}$ in different environments of Saskatchewan, applying N fertilizer to the inoculated plants affected yield only in the potentially low yield environments (Cowell et al., 1989). Gan et al. (2005) found that applying more than 15 kg N $ha^{-1}$ reduced both nodule number and nodule mass of inoculated lentil. Comparisons of fertilized and non-fertilized legumes for $N_2$ fixation must be interpreted cautiously when $N_2$ fixation data are estimated by the $\delta^{15}N$ abundance method (Unkovich and Pate, 2000), because the origin of N fertilizer is also atmospheric $N_2$. In this method, the proportion of plant $^{14}N/^{15}N$ is used to estimate the quantity of $N_2$ fixation. In western Canada, producers grow lentil, chickpea and pea using commercial rhizobium inoculants for $N_2$ fixation, and they don't apply N fertilizer, even a starter amount.

### 4.5.2 Seed yield

Nitrogen fertilizer did not reduce lentil yield in any environment, because the fertilized lentil accumulated as much N as did inoculated lentil. Although lentil seemed to be N-deficient in the control treatment, especially at SKA, the lowered yield was entirely independent of indeterminate growth of lentil. Earlier maturity of chickpea in response to N fertilizer in semi-aid environments of Saskatchewan lowered the yield, because the greatest yield belonged to treatments having the longest days to maturity (136d) in 2004 (Gan, et al., 2009a). This is in agreement with lentil yield at IH, where yield decreased in the control plots due to low $N_2$ fixation and low plant N content (Table 4.4). Variation in plant density may alter the ratio of primary/higher order branches and impact yield (Erskine and Goodrich, 1991), this variation was not included in data analysis, similar to the work by Gan et al., (2005). Wide ranges in plant population density of 0.1 to 4 times of the normal population did not impact average seed and plant N content (g $m^{-2}$) of chickpea, lentil, lupin (*Lupinus perennis*) and field pea in dry or wet years (Ayaz et al., 2004).

### 4.5.3 Cultivar responses

Lentil cultivars had different growth, N uptake, and yield, but their differences were not as substantial as findings of previous studies (Whitehead et al., 2000; Kurdali et al., 1997). The lowest yield came from CDC Blaze, along with lower DW and N yield. At SKA, CDC Blaze produced only two thirds of the highest yielding cultivar, 86% of the greatest DW, and 60% of the highest amount of fixed $N_2$. The low yield of CDC Blaze SKA was associated with low N availability at the location, the earlier maturity and a low $N_2$ fixation of this cultivar. Performance of lentil cultivars was independent of seed size and market class. At SKA, CDC Sedley, a large-seeded cultivar, produced lower yield, than most cultivars (Table 4.5); it also had the lowest plant N concentration (1.9%), total plant N and total fixed $N_2$ amongst the cultivars (Table 4.6). Two other large-seeded cultivars, CDC Plato and CDC Greenland, tended to have the greatest seed yield among the cultivars. Variation of $N_2$ fixation among cultivars seemed to be related to cultivar DW, because the greatest fixed $N_2$ belonged to the large-seeded CDC Greenland and CDC Plato, cultivars which produced larger plants. CDC Greenland and Red Rider yielded similarly to CDC Plato at GD and SKA, due to wider adaptability.

### 4.6 Conclusion

The findings of this study did not provide sufficient evidence to support the hypothesis of maturity induction via N fertilizer application to uninoculated lentil. The results further demonstrated that variability in yield and total production of lentil among years/locations in Saskatchewan was associated with rainfall and temperature patterns during the lentil growing season. Inoculated lentil produced a higher yield than non-inoculated lentil and tended to have a higher yield than the fertilized lentil. Improved $N_2$ fixation via inoculation with commercial rhizobia, which is cheaper and more sustainable than applying N fertilizer, could ensure greater yield under both suitable and limited conditions of moisture availability. In contrast, benefits of applying starter N could be limited to soils with low available N at seeding time only.

The large-seeded cultivars with greater potential of $N_2$ fixation, DW and N accumulation (CDC Plato, CDC Greenland, and occasionally CDC Sedley) yielded more than the small-seeded cultivars. CDC Milestone, small-seeded, which produced as much yield as the large-seeded cultivars and had reasonable days to maturity, may be a potential candidate for short growing seasons. CDC Red Rider and CDC Greenland had a wide range of adaptability to varied weather conditions, and they produced great yield in both suitable (GD) and drought (SKA) environments.

Further investigation on growth inhibitor agents, as a method of maturity induction in lentil, is recommended. Also, understanding the physiology of flowering and podding, under long days, cool temperatures, and short growing seasons of Saskatchewan could better direct the selection of cultivars with a shortened flowering span and more efficient reproductive growth period.

# 5.0 Lentil Response to Varied N Sources II. Above Ground Biomass and N Partitioning

## 5.1 Abstract

Leaf and stem production in lentil (*Lens culinaris* Medic.) may be reduced when N is in short supply. Applying N fertilizer can inhibit $N_2$ fixation and improve partitioning of carbohydrate and N to pods and not vegetative organs in this indeterminate pulse crop. The accumulated dry matter (DW) and N in leaf, stem and pod in eight cultivars of lentil were studied under three treatments of 50 kg N fertilizer $ha^{-1}$, granular rhizobium and a non-treated control in Saskatoon, SK in 2006 and 2007. Lentil matured by drought in both years, but it received more late-season rainfall and had access to more N in 2006.

By maturity, pods were 60% of total DW and 78% of total N, and stem and leaf shared the remaining portions. When more water was available in 2006, prolonged $N_2$ fixation and leaf production maximized pod DW and pod N in the inoculated lentil. In contrast, drought stress resulted in lack of N translocation from stem to pod in 2007. Compared to stem, leaf DW and N had greater contribution to yield formation by more translocation of N to seeds. Cultivar CDC Milestone accumulated more DW and N in pod than the other cultivars. Two large-seeded cultivars, CDC Plato and CDC Sedley, had the greatest and the smallest amounts of N remobilization from stem, respectively. Results suggested that further yield improvement in lentil is likely by selecting cultivars for greater stem N prior to pod set (as in CDC Sedley) and higher N remobilization from stem during the seed-filling period (as in CDC Blaze and CDC Plato).

## 5.2 Introduction

Seed yield in field crops is determined by biomass accumulation, and then by partitioning of the biomass to seed. Efficiency of the carbohydrate partitioning to seed, harvest index (HI), is linked to plant N status, because of long-term simultaneous selection for yield, protein and HI (Sinclair, 1998). The association of yield-plant N is more important in legume crops, where seed N requirement is substantial (Sinclair, 1998; Singh, 1977; Sinclair and de Wit, 1975). However, yield in legumes is less likely to be restricted by N, because these crops can fix most of their N requirements through atmospheric $N_2$ (Kurdali et al., 1997; van Kessel, 1994; Rennie and Dubetz, 1986).

Compared to non-legumes, $N_2$-fixing crop are rich in N. However, greater plant N may not necessarily translate to higher seed yield (van Kessel, 1994), possibly because of competition of vegetative organs and nodules with seeds for carbohydrate and N (Singh, 1997; Khatun et al., 2010). Competition of vegetative organs with seeds is more likely in legumes with indeterminate growth, in which leaf and stem production can be prolonged until maturity. For example, in the study of van Kessel (1994) lentil stems continued to compete for N with seed until maturity, whereas wheat (*Triticum aestivum* L.) stem supplied most of seed N requirement via remobilization. Similarly, chickpea (*Cicer arietinum* L.) continued to accumulate N in stem until maturity in dry conditions of Syria (Kurdali et al., 1997).

Lentil is a genetically indeterminate pulse crop; however, its indeterminate growth is inhibited by drought in most places (Kurdali, 1996; Silim et al., 2008). The post-flowering growth of indeterminate crops can be stimulated with more water during seed filling. Lentil matured late, produced substantially more biomass (up to 2000 kg $ha^{-1}$), but it had low yield and low HI in cool-wet environments of UK compared to a warm-dry growing season of Pullman, WA (Whitehead et al., 2000). In Saskatchewan, variation in rainfall and temperature between two years resulted in varied HI, seed N and seed yield in lentil (Malhi et al., 2007a). In New Zealand, lentil had low seed yield and HI in irrigated plots, compared to lentil grown under rainfed conditions in a dry year (McKenzie and Hill, 1990).

Plant N status is also known to stimulate vegetative growth and reduce partitioning of carbohydrate and N to seed. Lentil had more biomass, but lowered HI with N fertilizer than with inoculant rhizobia (Bremer et al., 1989). In the cool-wet conditions of UK, where lentil accumulated substantial N in a large biomass, HI and yield were both limited by insufficient seed N (Whitehead et al., 2000). Seed N restriction is more likely in the northern latitudes with cool temperature during seed-filling period (Larmure et al., 2005; Miller et al., 2002). Lack of strong correlations between HI with pod number (28%), 100-seed weight (-37%) and even seed yield (50%), and strong association of biomass and yield in lentil in the study of Singh (1977) may suggest inefficient partitioning of carbohydrate and N to seed in lentil.

In dryland cropping systems, where soil moisture in unmanageable, less available N to plants may limit vegetative growth and improve partitioning of N and DW to seeds. In soybean (*Glycine max* L.), lack of sufficient N initiated N remobilization from leaves and hastened maturity of an indeterminate cultivars (Munier-Jolain et al., 1994). Opposite results were found by applying 150 kg N $ha^{-1}$ to indeterminate soybean (Wallace et al., 1990). In semi-arid conditions of the northern Great Plains, applying N fertilizer, instead of inoculant rhizobia, could inhibit $N_2$ fixation and induce early maturity (Gan et al., 2009a).

Lentil is extensively grown in Saskatchewan, where late-summer rainfall and cool temperature during yield formation can stimulate vegetative growth and lower HI and yield. Under such circumstances, N and carbohydrate partitioning to seed may be affected by availability of N to plants. Limited $N_2$ fixation can reduce late season growth and improve HI by trigging N remobilization from leaf and stem. In contrast, late-season $N_2$ fixation may favor of leaf and stem expansion. However, due to strong correlation of plant N and seed yield, final yield can be unpredictable. This study hypothesized that applying 50 kg N fertilizer $ha^{-1}$ versus granular rhizobium inoculant can reduce $N_2$ fixation of lentil and, thereby, improve partitioning of biomass and N to seed; while, final pod DW and N may remain unaffected. Lentil grown in non-inoculated control without N fertilizer will have low biomass and yield, but improved HI.

The main objectives of this study were to:

*1) To measure leaf, stem and pod DW and N contents of lentil in three stages of flowering, mid-pod and maturity under three varied fertility treatments and environments,*

*2) To compare the accumulation and distribution of DW and N within the plant for eight lentil genotypes.*

### 5.3 Materials and methods

#### 5.3.1 Environment and cultivars

Field experiments were conducted in two locations at Saskatoon, SK (52º N and 106º W) in 2006 and 2007. The two locations were Goodale research farm in 2006 (GD) and Skarsgard research farm in 2007 (SKA), both located within 30 km radius of Saskatoon. The locations are alternatively referred to as environments and years throughout this Chapter. Spring soil test values of the fields are presented in Table 4.1 (Chapter 4) and average monthly temperature and total monthly rainfall of Saskatoon in 2006 and 2007 is presented in Table 4.2 (Chapter 4). Eight cultivars of lentil were grown under three fertility treatments of 50 kg N $ha^{-1}$, a commercial inoculant containing rhizobium, and an untreated control in both GD and SKA. Names of cultivars, seeding dates, plot maintenance, and experimental design are found in Chapter 4.

### 5.3.2 Sampling

Five lentil plants were sampled from each plot at three stages of flowering, mid-pod and maturity, as described in Chapter 4. The samples were refrigerated, and then separated into three fractions of leaf, stem and pod at the earliest opportunity within one to two days after sampling. The separated portions were oven dried at 60°C for 24 hr, weighed and fine ground. A sub-sample of the ground materials was analyzed for plant N concentration by combustion (LECO CNS 2000, Joseph, MI, USA). Total plant N content of each portion was calculated as described under the general methods (Chapter 3).

### 5.3.3 Data analysis

Data were analyzed as a nested design in Proc Mixed of SAS, version 9.2 (SAS Institute, Cary, NC) for each location separately. Weather conditions, especially rainfall distribution, varied between two years of study (see Table 4.2, Chapter 4). Also, soil cultivation history and soil N content affected response of lentil to the fertility treatments. Combined analysis of variance for two environments would skew the results, because of substantial variation between the environments. For analysis of variance, the main plots were fertility treatments, sub plots were cultivar, and sub-sub-plots were growth stages, with replication as the random term. Mean separation was done by LSD, $P < 0.05$.

## 5.4 Results

### 5.4.1 Pattern of DW and N accumulation

Lentil DW and N accumulation were often synchronized for leaf and pod, but stem consistently had less N concentration, meaning that stem DW accumulation exceeded its N uptake (Fig 5.1). By flowering, lentil invested more biomass in leaf than in stem (0.27 and 0.17 g DW plant$^{-1}$ in leaves and stems, respectively). By this stage, the leaf proportion was more concentrated in N, and contained almost five folds more N than stem. As plants grew to midpod filling, total plant biomass was almost equally divided among leaf, stem and pod (0.87, 0.95 and 0.76 g DW plant$^{-1}$ in leaves, stems and pods, respectively); however, stem did not have as much N as did leaf and pod fractions. Consequently, the proportion of stem N in plant declined to 16% at mid-pod. From mid-pod to maturity, absolute leaf remained unchanged, but the proportion of leaf DW to total plant DW declined due to leaf loss, and substantial increase in pod DW portion. By maturity, the majority of lentil DW and N was accumulated in pods (3.82 g DW and 125 mg N plant$^{-1}$), and stem had the least DW. Maximum DW and N, which were both accumulated during mid-pod to maturity, was in favor of the pod, which had the greatest DW (60%) and N content (78%) of plant (Fig 5.1, 5.2).

From mid-pod to maturity, 100 mg N plant$^{-1}$ was accumulated in pods (Fig 5.2). This massive increase in pod N partially came from remobilization of N from vegetative biomass, because leaf and stem N content declined during the same period. However, the amount of remobilized N, compared to the N uptake and fixation during reproductive growth, was small. From mid-pod to maturity, lentil accumulated 6.3 g N $m^{-2}$. From this amount, 4.2 and 2.1 g N $m^{-2}$ came from fixation and uptake from soil, respectively (Fig 4.1, Chapter 4). Overall, lentil yield and pod formation depended on the late season growth and N accumulation. Late-season leaf production, when more water was available, did not seem to compete with seed for N; however, it may lower the available carbohydrate to pods.

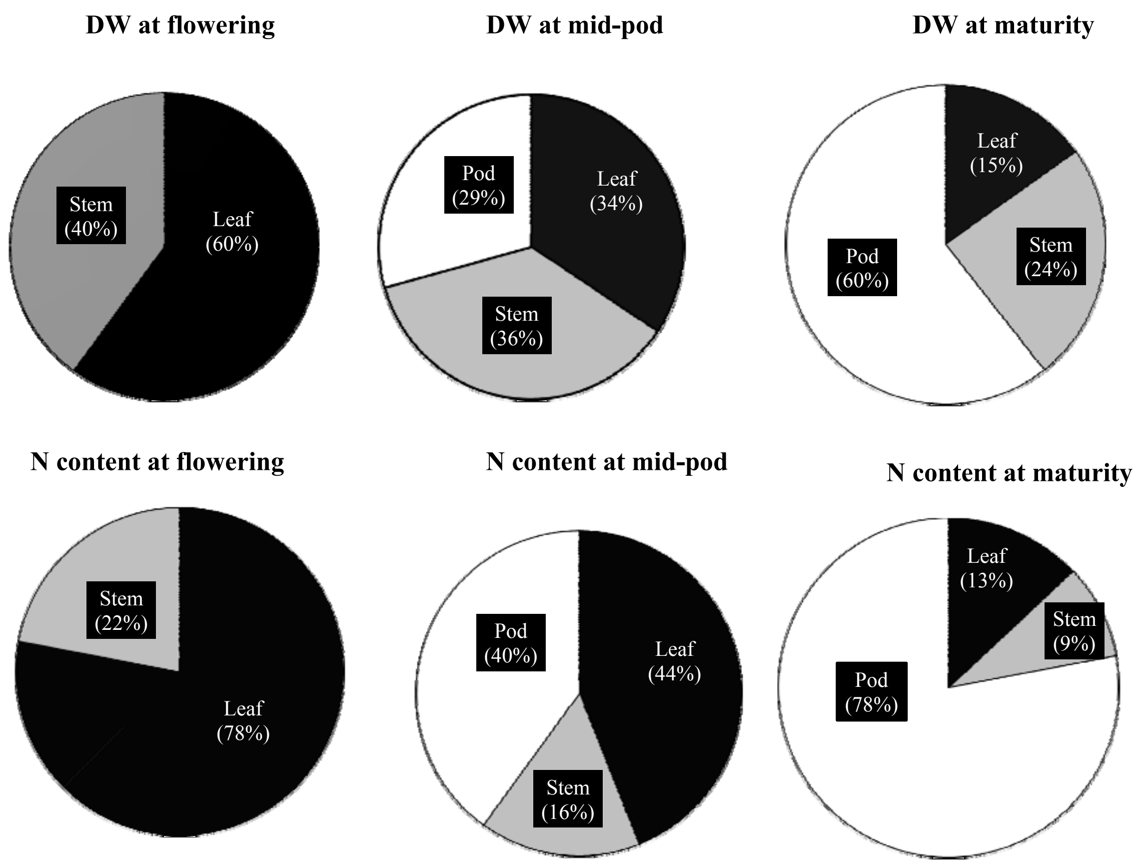


**Figure 5.1** Pattern of biomass (upper panel) and N (lower panel) partitioning among leaf, stem and pod during lentil growth. Data were averages over locations, treatments, cultivars and replications.

### 5.4.2 Analysis of variance

Separate analysis of variance for each environment showed that fertility treatments affected DW and N content of leaf, stem and pods at SKA only, where lentil growth and yield was restricted by drought, N deficiency and lack of effective indigenous rhizobia. At GD, average DW and N content of the lentil organs was independent of the N treatments; however interactions of treatments and growth stage was observed at this location (T× S in table 5.1). This interaction suggested that effects of the treatments on DW and N partitioning varied by growth stage. Cultivars differed for within plant partitioning of DW and N at both locations (Table 5.1). Again, interaction of cultivar and growth stage (C × S in Table 5.1) was observed for some variables at both locations.

**Table 5.1** Significant effects of the N fertility treatments , cultivars, growth stages (Stage) and their interactions on dry weight (DW) and N partitioning within plant in eight cultivars of lentil and two environments. Data were analyzed for each environment, separately.

| | | DW (g plant$^{-1}$) | | | | | | Total N (mg N plant$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Leaf | | Stem | | Pod | | Leaf | | Stem | | Pod | |
| **Effect** | **DF** | GD | SKA | GD | SKA | GD | SKA | GD | SKA | GD | SKA | GD | SKA |
| **N treatment (T)** | **2** | ns | ** | ns | ** | ns | * | ns | * | ns | ** | ns | ns |
| **Cultivar ( C)** | **7** | ** | ** | ** | ** | ns | ** | ** | ** | ** | ** | ns | ** |
| **T × C** | **14** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| **Stage (S)** | **2** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| **C × S** | **14** | ns | ns | ** | * | ns | ns | ns | ns | ns | ** | ns | ns |
| **T × S** | **4** | ns | ns | * | ** | ** | * | ns | * | ns | ** | * | * |
| **T × C × S** | **28** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

*, ** and ns indicate significant ($P<0.05$), highly significant ($P<0.01$) and not significant differences among treatments, cultivars, growth stages (sample) and their interactions within each environment

### 5.4.3 Environment-treatments effects

Lentil produced larger plants with greater stem, leaf and pod DW at GD than SKA. Similarly, N concentration and total N content of the three plant organs were greater at GD than SKA.

Average pod DW at maturity was 4.5 g plant$^{-1}$ at GD and 3.6 g plant$^{-1}$ at SKA (Fig 5.2). Overall soil and weather conditions at GD were similar to normal growing conditions of lentil in the Brown Soils of Saskatchewan, but lentil in the SKA was limited by unusual drought and soil N deficiency (see Table 4.2, and Fig. 4.1 in Chapter 4).

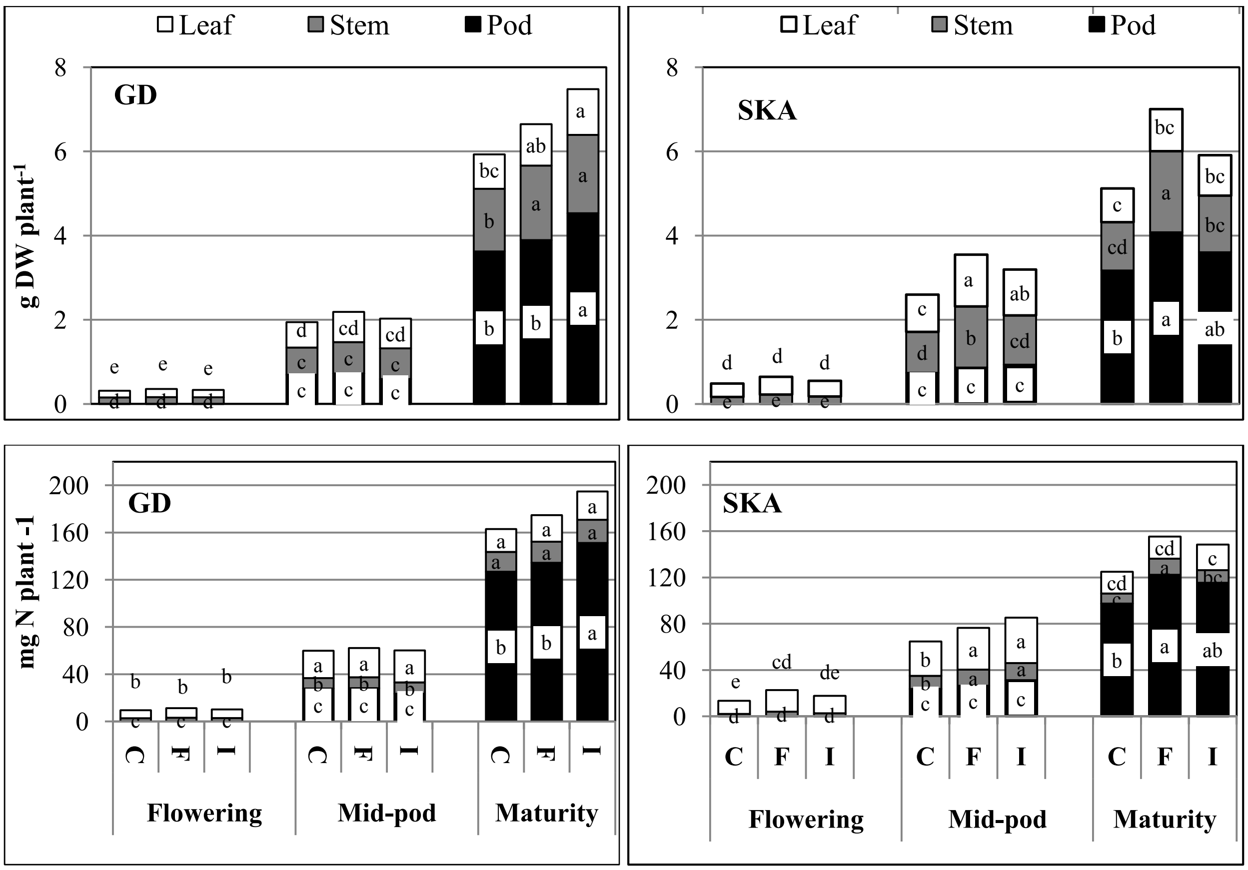


**Figure 5.2** Significant effects of the N fertility treatments (C: Control, F: N fertilizer and I: Inoculant) and growth stage at sampling on the partitioning of biomass (upper panel) and N (lower panel) at GD and SKA. Within each partition of leaf, stem or pod for all stages, means followed by the same letter do not differ at P<0.05.

Effects of the fertility treatments on partitioning of DW and N within plant organs differed by environment. At GD, the fertility treatments affected leaf, stem and pod DW and N content of the lentil cultivars only at physiological maturity. Lentil grown under the inoculant and N fertilizer treatments produced more leaf, stem and pod DW than lentil in the untreated control. Pod DW and pod N were both maximized in the inoculated lentil (Fig 5.2), suggesting that the post-flowering leaf and stem production could not compete for N with pods.

Slightly greater leaf DW in the inoculated lentil than in the other two treatments by maturity at GD may be associated with late season $N_2$ fixation, which somewhat improved the pod proportion (Table 5.3). Despite varied leaf, stem and pod DW values by the fertility treatments, the proportion of these organs in the final plant biomass remained independent of the treatments, indicating that total plant N had greater association with yield than partitioning of N within lentil plants. At GD, stem and leaf N became diluted as lentil grew, likely because of both N remobilization and biomass expansion. The small N content of leaf and stem compared to pod at maturity demonstrated that pods effectively competed with vegetative biomass for the N. Photosynthesis efficiency in the N-deficient leaves (and stems) was expected to reduce by this time, yet they could compete with seeds for carbohydrate.

Different from GD, fertility treatments affected both absolute and the proportion of lentil stem, leaf and pod DW and N in plant at SKA, more likely due to drought stress. At this location, effects of fertility treatments on lentil appeared at early growth of lentil. By flowering, lentil had similar leaf and stem DW across the fertility treatments, but leaf in the fertilized lentil was more enriched in N than in the other two treatments (Fig 5.2). Great leaf N concentration in the fertilizer treatment compared to the other treatments also indicated that soil N-deficiency restricted early growth of lentil at SKA (data not presented).

At mid-pod, which leaf DW was still identical across the treatments at SKA, stem DW was maximized by the N fertilizer treatment, and pod DW was maximized by both the N fertilizer and the inoculant treatments (Fig 5.2). By maturity, when plants had experienced a severe drought at SAK, leaf DW and N did not differ by the fertility treatments, but stem DW and N, and pod DW and N were greater in the treated plots than in the control. At maturity, lentil grown at SKA produced 4.5, 3.9 and 3.6 g pod DW plant$^{-1}$ and had 151, 134 and 127 mg pod N plant$^{-1}$ in response to inoculant, fertilizer and control treatments, respectively (Fig 5.2). These results are in agreement with the yield data, which lentil yielded more under both inoculant and fertilizer treatments than in the control. (Chapter 4, Table 4.5).

### 5.4.4 Dynamics of DW and N

During the seed-filling period, plants undergo carbohydrate and N remobilization from vegetative organs to seeds. At GD, leaf DW was increased by 44%, whereas leaf N content declined by 12% during seed-filling (Table 5.2). Among the treatments, leaf DW had a greater increase in the inoculant than in the fertilizer and control treatments, but leaf N declined across the three treatments at GD (Table 5.2). At this location, substantial increased in stem DW (160%) and stem N (96%) from mid-pod to maturity was independent from the fertility treatments. Different from GD, both leaf DW and leaf N was decreased during the seed-filling, due to drought stress of SKA. Again, stem DW slightly increased during the seed-filling, but stem N was depleted at the same time. At this location, stem N content remained constant in the fertilized lentil, but it declined by 15% in the other treatments during the yield formation (Table 5.2).

Table 5.2 Changes in leaf and stem DW and N content during the period mid-pod to maturity, the proportion of vegetative DW to total plant DW at maturity, and proportion of vegetative N to total plant N at maturity. A negative value indicates DW or N at maturity is lower than the mid-pod value, a positive value indicates DW or N at maturity is greater than the mid-pod value.

| | Changes in leaf DW %† | | Changes in stem DW %†† | | Veg DW to total plant DW %††† | |
|---|---|---|---|---|---|---|
| | **GD** | **SKA** | **GD** | **SKA** | **GD** | **SKA** |
| **Control** | 33 | -11 | 150 | 10 | 39 | 37 |
| **Fertilizer** | 43 | -17 | 157 | 27 | 42 | 41 |
| **Inoculant** | 57 | -9 | 171 | 8 | 40 | 39 |
| Averages | **44** | **-12** | **160** | **15** | **40** | **39** |
| | %Changes in leaf N † | | %Changes in stem N†† | | %Veg N/ Plant N††† | |
| | **GD** | **SKA** | **GD** | **SKA** | **GD** | **SKA** |
| **Control** | -17 | -37 | 89 | -18 | 22 | 22 |
| **Fertilizer** | -8 | -47 | 100 | 0 | 23 | 21 |
| **Inoculant** | -11 | -44 | 100 | -27 | 23 | 22 |
| Averages | **-12** | **-42** | **96** | **-15** | **23** | **22** |

† (leaf DW or N at maturity- leaf DW or N at mid-pod) x 100/ leaf DW or N at mid-pod
†† (stem DW or N at maturity- stem DW or N at mid-pod) x 100/ stem DW or N at mid-pod
††† (leaf DW or N + stem DW or N) x 100 / (total plant DW or N)

Despite substantial variation in the DW and N dynamics among environments and fertility treatments, the final proportions of vegetative biomass and N in plants were identical among treatments and environments (last two columns in Table 5.2). At maturity, 40% of plant biomass and 22% of plant N were accumulated in vegetative organs and the remained portions belonged to pods at both locations. Lentil growth was terminated by drought at both locations, and more late-season rainfall would probably stimulate growth and increase final proportion of vegetative biomass in the inoculated treatment.

Association of the three plant organs (leaf, stem and pod) with yield, HI and days to maturity varied by weather conditions and N availability. Accumulated N in vegetative biomass (leaf N and stem N) had stronger correlations with yield at SKA than at GD (Table 5.3). At GD, with greater end-of-season moisture, lentil appeared to supply the N in yield largely by current photosynthesis driving $N_2$ fixation and current N uptake. Harvest index was negatively affected by greater leaf and stem N content at GD, where available water and N improved the post-flowering vegetative biomass accumulation and yield of lentil (Table 5.3).

Plant photosynthesis is directly associated with leaf biomass and leaf N; however, the late season leaf production at GD competed with yield and had negative impacts on HI. Similarly, strong negative correlations of stem N with HI at GD (-55% at mid-pod, -41% at maturity) demonstrated that under favorable conditions of growth, stems became a strong sink for carbohydrate and N, and competed with seed. At SAK, greater pod DW was associated with greater leaf DW. Among the cultivars, CDC Greenland, CDC Milestone and CDC Plato had greater leaf DW and pod DW in the inoculant than in other treatments, (Fig 5.2). In the dry conditions of SKA leaf and stem maintenance during yield formation may extend the duration of active photosynthesis, N uptake and probably $N_2$ fixation and N remobilization to seed (Table 5.3)

Table 5.3 Correlation coefficients (P<0.05) of total N contents of leaf, stem and pod partitions with yield, HI and days to maturity for eight lentil cultivars at two growth stages. Non-significant coefficients (--) are not reported, N = 96.

| | Growth Stage | Seed yield | | Harvest Index | | Days to maturity | |
|---|---|---|---|---|---|---|---|
| | | GD | SKA | GD | SKA | GD | SKA |
| **Leaf N (g m$^{-2}$)** | Mid-pod | 0.41 | 0.48 | -0.39 | -- | 0.39 | 0.28 |
| | Maturity | -- | 0.32 | -0.43 | 0.25 | 0.36 | 0.42 |
| **Stem N (g m$^{-2}$)** | Mid-pod | -- | 0.51 | -0.55 | -- | 0.46 | 0.31 |
| | Maturity | -- | 0.37 | -0.41 | -- | 0.36 | -- |
| **Pod N (g m$^{-2}$)** | Mid-pod | 0.24 | 0.25 | 0.21 | 0.31 | -0.35 | -- |
| | Maturity | 0.64 | 0.79 | 0.45 | 0.64 | -- | -- |

-- Correlation coefficients were not significant

### 5.4.5 Cultivar effects

Cultivars differed for the partitioning of DW and N among leaf, stem and pod at both mid-pod and maturity and in both environments. Averaged over two locations at maturity, CDC

Greenland and CDC Plato had the greatest, and CDC Blaze had the smallest leaf and stem N, and other cultivars were in between. At both GD and SKA, CDC Blaze had the smallest leaf and N than other cultivars at maturity, possibly because of the earlier maturity of this cultivar (Table 5.4). All cultivars were sampled at a same time and earlier maturity of CDC Blaze may result in leaf loss. CDC Blaze had also one of the smallest stem DW and stem N by maturity among the cultivars at both locations (Table 4.5).

Table 5.4 Average total N content in leaf, stem and pod partitions of eight cultivars grown at GD and SKA. Data from measurements at mid-pod (P) and physiological maturity (M). Data are averaged over three fertility treatments and four replications, N=12.

| | **Leaf N content** | | | | **Stem N content** (mg N plant $^{-1}$) | | | | **Pod N content** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cultivar** | **GD** | | **SKA** | | **GD** | | **SKA** | | **GD** | | **SKA** | |
| | **P** | **M** | **P** | **M** | **P** | **M** | **P** | **M** | **P** | **M** | **P** | **M** |
| CDC Blaze | $19^{c}$ | $17^{bc}$ | $27^{b}$ | $16^{b}$ | $6^{c}$ | $12^{c}$ | $9^{d}$ | $6^{c}$ | $26^{bc}$ | $120^{bc}$ | $24^{bc}$ | $119^{abc}$ |
| CDC Rouleau | $24^{b}$ | $22^{abc}$ | $35^{b}$ | $17^{ab}$ | $9^{b}$ | $19^{ab}$ | $11^{bcd}$ | $10^{bc}$ | $29^{b}$ | $134^{ab}$ | $27^{bc}$ | $108^{bcd}$ |
| CDC Red Rider | $25^{ab}$ | $16^{c}$ | 32 | $21^{ab}$ | $10^{ab}$ | $17^{bc}$ | $14^{bc}$ | $12^{bc}$ | $22^{c}$ | $124^{bc}$ | $24^{bc}$ | $99^{cd}$ |
| CDC Milestone | $25^{b}$ | $20^{abc}$ | $37^{b}$ | $18^{ab}$ | $8^{b}$ | $14^{bc}$ | $13^{bcd}$ | $11^{bc}$ | $36^{a}$ | $144^{ab}$ | $38^{a}$ | $139^{a}$ |
| CDC Viceroy | $31^{a}$ | $25^{ab}$ | $28^{b}$ | $21^{ab}$ | $9^{b}$ | $18^{bc}$ | $10^{cd}$ | $8^{c}$ | $27^{bc}$ | $154^{a}$ | $18^{c}$ | $87^{d}$ |
| CDC Greenland | $29^{ab}$ | $27^{a}$ | $37^{b}$ | $22^{ab}$ | $12^{a}$ | $20^{ab}$ | $15^{b}$ | $13^{ab}$ | $21^{c}$ | $131^{b}$ | $29^{b}$ | $135^{ab}$ |
| CDC Plato | $25^{b}$ | $23^{abc}$ | $50^{a}$ | $24^{a}$ | $10^{ab}$ | $19^{ab}$ | $21^{a}$ | $11^{bc}$ | $24^{bc}$ | $140^{abc}$ | $31^{ab}$ | $104^{bcd}$ |
| CDC Sedley | $23^{bc}$ | $24^{abc}$ | $32^{b}$ | $19^{ab}$ | $10^{ab}$ | $25^{a}$ | $14^{b}$ | $9^{c}$ | $26^{bc}$ | $151^{a}$ | $25^{bc}$ | $102^{cd}$ |

Means followed by same letter within columns indicate non-significant differences among the cultivars at each growth stage and each environment ($P<0.05$)

Maximum pod N belonged to CDC Milestone and the smallest pod N belonged to CDC Red Rider; however, most of the differences for pod N were not significant (Table 5.4). Drought incidence at SKA reduced pod N of some cultivars, when it compared to lentil at GD. CDC Viceroy and CDC Sedley had the greatest reduction of pod N (58 g N plant$^{-1}$) due to the drought, whereas CDC Blaze, CDC Greenland and CDC Milestone had identical final pod N at both locations (Table 5.4). These cultivars may have some forms of drought resistance which helped them to maintain their pods under drought.

Ability of leaf maintenance from mid-pod to maturity varied among the cultivars and environments. At GD, where other cultivars lost 2 to 6 mg leaf N plant$^{-1}$, CDC Red Rider lost the greatest leaf N content (9 mg N plant$^{-1}$) and CDC Sedley gained 1 mg leaf N plant$^{-1}$ during the seed-filling (Table 5.4). In dry conditions of SKA, CDC Plato had the greatest loss of leaf N (26 mg N plant$^{-1}$) and CDC Viceroy had the smallest leaf N loss (7 mg N plant$^{-1}$) during the same period (Table 5.4, Fig. 5.3). Variation of leaf N by the environments showed that leaf N remobilization was negligible when soil had more moisture.

Maximum stem N accumulation (15 mg N plant$^{-1}$) belonged to CDC Sedley at GD, where other cultivars gained a range of 6-9 mg stem N plant$^{-1}$ during the lentil seed-filling (Table 5.4). At SKA, CDC Plato lost more N from stem (10 mg N plant$^{-1}$) than other cultivars that lost 2 to 5 mg N plant$^{-1}$ from stem during seed-filling (Table 5.4, Fig. 5.3). Pod N content represented the majority of plant N by maturity in the lentil cultivars (Table 5.2, Fig. 5.1 to 5.3). CDC Milestone accumulated the greatest amount of pod N by mid-pod at both GD (36 mg N plant$^{-1}$) and SKA (38 mg N plant$^{-1}$). It also had one of the greatest amounts of total plant N (Chapter 4, Table 4.4) among the cultivars by maturity at both GD (144 mg N plant$^{-1}$) and SKA (139 mg N plant$^{-1}$).

Interactions of the fertility treatments by growth stages and cultivars by growth stages at SKA in Table 5.1 was associated with varied response of large and small seeded cultivars to N deficiency. For example, CDC Blaze had as much pod DW and pod N as the other cultivars in the control treatment. However, with more N availability in the N fertilizer and inoculant treatments, CDC Milestone and CDC Greenland maximized their pod N, whereas CDC Blaze had lower pod N. Similarly, leaf N in the large-seeded cultivars responded better to the fertility treatments than the leaf N in the small-seeded cultivars (Table 5.4). Averaged over GD and SKA, CDC Greenland had 9 and CDC Plato had 14 mg plant$^{-1}$ greater leaf N in the inoculant treatment than in the other two treatments (data not shown). These cultivars produced 55 and 20 mg greater pod DW plant$^{-1}$, respectively, in the inoculant compared to the other two treatments (Table 5.4).

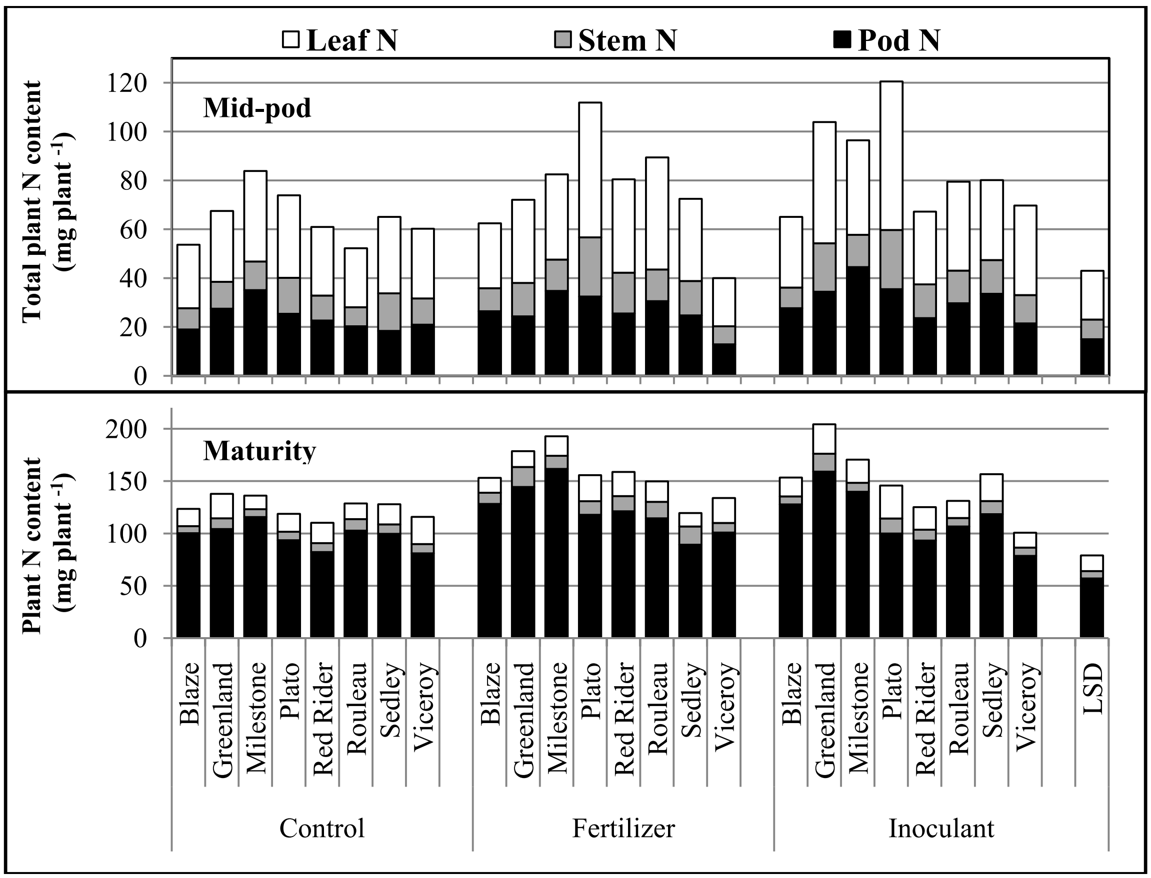


Figure 5.3 Average leaf N, stem N and pod N content of eight lentil cultivars grown at N limited and dry conditions of SKA at mid-pod(upper panel) and maturity (lower panel). Each bar chart is a mean of four replications.

### 5.5 Discussion

Both dry matter and total N mass of lentil were constantly increased throughout the growing season. Averaged over the two years, total plant DW was increased from ~ 2 g plant$^{-1}$ at mid-pod to about 6 g plant$^{-1}$ at maturity (was almost 300% increase in DW). Simultaneously, total plant N content was increased by about 150%. The substantial increased DW and N were both in favor of pod proportion, which gained more than 2.5 g DW plant$^{-1}$ and 80 mg N plant$^{-1}$ from mid-pod to maturity (Fig. 5.1, 5.2). This pattern of growth and N uptake demonstrated that lentil relied on late season growth and $N_2$ fixation or N uptake for growth and yield. These findings are in agreement with results of studies by Whitehead et al. (2000); van Kessel (1994); Kurdali et al. (1997) and Singh (1977), which all indicated the association yield and biomass in lentil. In the van Kessel (1994) study, about 50% of total biomass and about 40% of total N in lentil (67 kg N ha$^{-1}$) was accumulated from 71 days after seeding [around first to mid-pod] to maturity, during the time that seeds obtained 70 kg N ha$^{-1}$.

Fertility treatments did not alter partitioning of DW and N to lentil seeds when adequate rainfall followed by a terminal drought at GD. At SKA, slightly effects of the treatments on the within plant partitioning of DW and N was associated with drought. The proportion of plant organs DW and N would have different responses to the fertility treatments with higher late-season rainfall. For example, rainfall and temperature variations resulted in substantial fluctuations in HI, yield and seed N content of two lentil cultivars in a two-year study in Saskatchewan (Malhi et al., 2007a). Likewise, more late-season rainfall stimulated growth and reduced HI and yield of lentil in two separate studies by Whitehead et al. (2000) and McKenzie and Hill (1990).

In addition, the fertilizer treatment failed to impact total plant N content. Thus, partitioning of DW and N among the lentil plant organs was identical in the fertilizer and inoculant treatments. In contrast to these results, applying 400 kg N fertilizer $ha^{-1}$ stimulated vegetative biomass and lowered HI of field pea (*Pisum sativum* L.); however, yield was not affected (Voisin et al., 2002). In soybean, applying 150 kg N $ha^{-1}$ to an indeterminate cultivar increased branch length and reduced yield via lodging and infested diseases (Wallace et al., 1990). Based on the study results, the main hypothesis that late season N deficiency improve partitioning of DW and N to pods was rejected. Both the proportion and absolute amounts of DW and N in pods were improved by more available N in the fertilizer and inoculant treatments. In contrast, leaf, stem and pod DW and N were all restricted by lack of sufficient N in the control. Similar to the performance of lentil in the control treatment, limited plant N reduced seed N concentration of field pea in the study of Lhuillier-Soundélé et al., (1999). In soybean, seed growth was terminated earlier in N-deficient plots than in plots received N fertilizer during the plant yield formation (Munier-Jolain et al., 1996).

Under severe drought conditions of SKA, leaf and stem production positively increased HI and yield of lentil. Longer leaf maintenance under drought may prolong photosynthesis, $N_2$ fixation and carbohydrate-N remobilization to seeds. In chickpea, osmotic adjustment extended leaf greenness and improved yield under terminal drought (Turner et al., 2007). Although correlation analysis did not show any association between leaf and stem production and seed yield at GD (Table 5.2), both pod DW and pod N were maximized when leaf production was high (Fig 5.2). The association of seed yield and biomass in lentil was previously indicated in other researches (Whitehead et al., 2000; Kurdali et al., 1997; Malhi et al., 2007a).

Compared to stem, leaf effectively contributed to yield formation via increased N accumulation at early season and promoted N remobilization toward maturity (Fig 5.1, Table 5.3). Stem gained more DW, but consistently had lower N than leaves, suggesting lower photosynthesis. Similarly, van Kessel, (1994) found that lentil leaf supplied 17 kg N $ha^{-1}$ to seed via remobilization, whereas stem N continued to increase until maturity. In chickpea, another indeterminate pulse crop, stem continued to accumulate N until maturity (Kurdali, 1996). In contrast, final stem N was greater than leaf N in the study of Whitehead et al. (2000). However, the N partitioning in their study was limited to a warm-dry environment, where lentil lost up to 861 kg biomass $ha^{-1}$ and up to 23 kg N $ha^{-1}$ during seed-filling.

The greater stem DW in the fertilized than non-fertilized lentil at SKA was explained by the early-season available N in the fertilizer treatment, which could increase leaf and stem DW. Later, rapid drought and heat stresses resulted in leaf loss, while stem DW was unaffected. Overall results of this study from both environments suggested that stem N capacity prior to pod set was low (compare leaf N versus stem N portion in Fig. 5.1). In the Mediterranean climate of Syria and under rainfed conditions, 86% of seed N of lentil was provided via remobilization from shoot and root; however the proportion of each organ was not indicated (Kurdali et al., 1997).

Reduction in DW and N during the period of mid-pod to maturity could address variation of cultivars for ability of N and carbohydrate remobilization to seed. Part of the reductions in leaf DW and N was related to leaf loss, but reduction in stem N was certainly associated with N remobilization, because stem breakdown is not common in lentil. Monitoring plant N concentration throughout plant growth may provide close estimates of DW and N remobilization during seed filling (Schiltz et al., 2005). However, in an indeterminate crop such as lentil, remobilized carbohydrate and N from older biomass may not necessarily be transferred solely to seed due to continuous vegetative growth. Monitoring N concentration of the most recent leaves in lentil during the growing season demonstrated that late-season leaves were depleted in N (see Chapter 8). Remobilization measurements by tracking plant dry weight and N status throughout the growing season may mislead the results in legume crops, with simultaneous biomass loss and biomass production during the growing season (Hay, 1995). Direct tracking of N during the growing season, by isotope method, accurately determined that field pea obtained 30%, 20%, 11% and 10% of its total seed N from remobilized N from leaves, pod walls, roots and stems, respectively (Schiltz et al., 2005).

Small-seeded cultivars are slightly more efficient than large-seeded cultivars in their partitioning of carbohydrate and N to pod. Under both environments, the greatest ratio of harvested N to pods, *i.e.* the proportion of pod N to vegetative N, belonged to CDC Blaze and CDC Milestone. However, the efficiency of partitioning of N to pod was translated to greater pod N in CDC Milestone only. In dry conditions of Syria, Kurdali et al. (1997) measured greater N remobilization in a small-seeded cultivar than in a large-seeded cultivar. Averaged over three rainfed environments in Syria, large-seeded cultivars developed stronger association between biomass and yield than small seeded cultivars (Erskine and El Ashkar, 1993). In Bangladesh, lentil produced larger seed size at early season than in late season (Khatun et al., 2010). Slightly greater pod N proportion in drier conditions of SAK than GD may result from the effect of the time of seed formation on seed size.

At both locations, the effects of N availability on vegetative biomass and N content of leaf, stem and pod appeared after plant flowering and continued until maturity, suggesting that pre-flowering measurements could be omitted. Furthermore, most cultivars did not show substantial variation for accumulating and partitioning of DW and N. In future studies, cultivar selection must be limited to those which are different for growth, days to flowering and days to maturity.

**5.6 Conclusion**

Most lentil pod DW and N was supplied via continuous growth and N uptake and $N_2$ fixation during seed-filling, demonstrating that lowered soil N could reduce vegetative growth and, thereby, pod DW and pod N. Furthermore, prolonged N supply in the inoculated lentil at GD improved pod DW and N, compared to the other treatments. Although inoculation of soil with rhizobia may not necessarily increase yield in lentil (Chapter 4), it could affect on other aspects of growth. Soil and weather conditions of GD were almost similar to soil conditions and long-term weather averages in the Brown soil zone of Saskatchewan. Under these circumstances, effective N supply toward maturity would not lower lentil HI and yield.

Although stem DW continued to increase during seed-filling at GD, it did not accumulate noticeable N, and stem remained depleted in N. In addition, final stem DW and N did not differ by the fertility treatments, suggesting low capacity of lentil stem for gaining N. In this scenario, cultivars with greater stem DW and N capacity prior to pod set, and more carbohydrate and N remobilization during seed-fill may further improve lentil yield. CDC Sedley had the greatest amounts of stem N, but it failed to transfer the N to pods. In contrast, CDC Plato transferred more N from stem to pods compared to most large-seeded cultivars. CDC Milestone showed the potential of sufficient DW and N accumulation and efficient partition of DW and N to seeds. Small-seeded cultivars may be considered for the short-season areas. However, results of this research could not identify any advantages of higher carbohydrate and N partitioning to seed in the small-seeded cultivars.

Finding the source of carbohydrate and N for the newly grown leaves and stems under suitable conditions of moisture could facilitate future breeding for more efficient growth under cool temperature. Further investigations on possible relation between seed position and yield with plant N and carbohydrate status are suggested.  In future, a study of DW and N partitioning alongside the plant height may address the most important part of canopy in yield formation of lentil.

# 6.0 Lentil Growth and N Accumulation as a Function of No-Till

## 6.1 Abstract

Improved soil N availability during the course of no tillage (NT) can lead to excessive vegetative growth of and low yield of the indeterminate crop, lentil (*Lens culinaris* Medic.). Experiments were conducted to investigate the effect of NT duration on lentil performance in the Black Soil Zone of Saskatchewan at Indian Head from 2006 to 2008. Five cultivars were grown in two fields with short (SN: 5 yr) and long (LN: 25 yr) histories of NT. A second experiment tested the effects of N fertilizer on one cultivar, CDC Sedley, also under both SN and LN. Averaged over the five cultivars, above ground biomass (DW), total N and yield of the lentil cultivars in SN was greater than in LN in 2006, when lentil growth was terminated by a mild drought. In that year, greater yield of CDC Sedley in SN than in LN disappeared by applying 60 kg N $ha^{-1}$. Late-season rainfall and cool temperature altered the response of some (2007) or all cultivars (2008) to NT duration, compared to 2006. Again, applying N fertilizer to CDC Sedley reduced differences between SN and LN in the cool-wet conditions of 2008, when CDC Sedley produced more yield in LN than in SN. Nitrogen fixation measured in 2008 showed greater %*Ndfa* for the five lentil cultivars in SN than in LN. Cultivars CDC Milestone and CDC Robin accumulated more N, had greater HI and produced more yield than other cultivars, likely because of greater $N_2$ fixation. The impact of late season N on lentil has a variable response depending on water and temperature. Late season N can increase yield when drought terminates the crop, but can exacerbate vegetative growth in long seasons.

## 6.2 Introduction

Late-season rainfall can stimulate vegetative growth, delay maturity, and lower HI and yield of lentil (McKenzie and Hill, 1995). It can also increase the risk of fall-frost damage in some years in the northern Great Plains (Miller et al., 2002). Similar to other indeterminate crops, post-flowering growth of lentil can be further stimulated with excessive N (Munier-Jolain et al., 1996, Wallace et al., 1990), available in the NT farms in the Black Soil Zone of western Canada (Schoenau et al., 2008; Sharifi et al., 2008). In addition, improved soil moisture under long-term NT management can increase $N_2$ fixation of pulse crops (van Kessel and Hartley, 2000), whereas high levels of soil N can potentially interfere with $N_2$ fixation (Luciñski et al., 2002; Matus, 1997). Considering cumulative effects of NT practice on soil properties over time, continual N build up during the course of NT is likely to interfere with vegetative growth, $N_2$ fixation and yield of lentil.

Numerous studies in the northern Great Plains have addressed the continual increase of soil carbon and N in response to continuous NT. In Saskatchewan, Schoenau et al. (2008) measured greater N in a soil with long-term NT (25-year) than with short-term NT (5-year) management. In a low-organic matter soil, corn yield in NT steadily increased with time and became similar or exceeded yield in conventional tillage (CT) because of improved soil conditions (Griffith et al., 1988). In the study of Lupwayi et al., (1998) concentrations of carbon and N were both increased during the course of NT, whereas soil pH and soil salinity declined. By the end of a long-term (11 years) study in southwestern Saskatchewan, Campbell et al. (1996) measured 3 tonnes greater carbon $ha^{-1}$ in NT than in CT.

Improved crop yield due to NT practice can substantially vary due to weather (Cutforth et al., 2006; Malhi et al., 2001); the magnitude of effect of NT on yield also varies among crops (Malhi and Lemke, 2007; Lafond and Derksen, 1996). In a cool-wet year in the semi-arid conditions of south western Saskatchewan, growing chickpea (*Cicer arietinum* L.) on chemical fallow resulted in delayed maturity compared to growing on continuously cropped cereal stubble. Delayed maturity and lowered seed yield were attributed to more available moisture and greater residual N in the fallow (Gan et al., 2009b). Moderate rates of N fertilizer inhibited $N_2$ fixation of chickpea and hastened maturity of this indeterminate crop (Gan et al., 2009a).

Delayed maturity of indeterminate soybean (*Glycin max* L.) in response to N fertilizer was attributed to delayed N remobilization from leaves, which would trigger onset of the leaf senescence and plant maturity (Munier-Jolain et al., 1996). Compared to the drought conditions of Syria (Kurdali et al., 1997), cool weather combined with greater N under irrigated conditions in western Canada resulted in poor remobilization of N in lentil (van Kessel, 1994). Lowered HI and yield of indeterminate crops with more N and water is also likely (Wallace et al., 1990). In Saskatchewan, applying N fertilizer increase vegetative biomass, but may not affect yield, resulting in lowered HI (Bremer et al., 1989).

Given that continuous NT increases soil available N and water, effects of NT on growth, N accumulation and yield of lentil may differ as the NT system ages. Lentil is expected to have different performances in fields subjected to varied durations of NT practice. Better soil N and moisture with prolonged NT can increase lentil growth and affect yield, whereas it can negatively interfere with $N_2$ fixation. Under these circumstances, response of lentil growth and yield to the duration of NT to additional N fertilizer is unpredictable, because the added N can compensate for N shortage in shorter NT, or can lower $N_2$ fixation with longer NT. This study aimed to compare the response of lentil in terms of growth, yield and N uptake to two lengths of 5 or 20 years NT. Also, effects of N fertilizer on the response of lentil to the duration of NT were investigated to determine if N deficiency or N excess was playing a role.

## 6.3 Materials and Methods

### 6.3.1 Experiment setup

Experiments were carried out in two adjacent fields, ~200 m apart, with either long-term (LN) or short-term (SN) histories of NT in the thin-Black Soil Zone of Saskatchewan at Indian Head (50° N and 103° W), during 2006-2008. By 2006, the LN field had been in continuous NT for 28 yr and the SN field had been in continuous NT for 5 yr. The SN plots were in a slightly lower elevation than LN plots. Both sets of plots had a very slight western aspect. Average soil chemical properties over the three years for each NT system are presented in Table 6.1. Soil samples were taken from 0 to 30 cm of each plot at spring (one to two weeks after seeding) and fall (at physiological maturity) and analyzed for $NO_3^-$ and $NH_4^+$ by a 2M KCL extraction method as described in Keeney and Nelson (1982).

Table 6.1 Soil chemical properties averaged over three years, in short-term (SN) and long-term (LN) no tillage fields. Soil samples were taken from the depth of 0-30 cm from all plots.

| Sampling Time | Tillage system | $NO_3^-$ | $NH_4^+$ | P† | K† | pH† | EC† | Carbon† |
|---|---|---|---|---|---|---|---|---|
| | | µg g$^{-1}$ | | | | | µmos cm$^{-1}$ | % |
| Spring | SN | 5.9* | 2.4 | 8.3 | 152 | 7.9 | 0.25 | --- |
| | LN | 9.1* | 2.2 | 9.1 | 422 | 7.7 | 0.21 | --- |
| Fall | SN | 2.4 | 2.8 | 10.8 | 267 | 7.7 | 0.16 | 1.8 |
| | LN | 2.5 | 2.7 | 22.3 | 434 | 6.9 | 0.14 | 2.2 |

*Indicates significant (P<0.01) differences of between two tillage systems
†Data were not analyzed, due to limit number of samples

Two separate studies were designed in each NT system of LN and SN: a cultivar study and a N fertilizer study. In the cultivar study, five cultivars of lentil, CDC Sedley (large green-seeded market class), CDC Milestone and CDC Vantage (small green market class) and CDC Robin and Redcap (small red market class) were planted in three replications in both LN and SN locations. In 2008, one plot of barley (*Hordeum vulgare* L.), cultivar AC Metcalfe, was seeded alongside each replication of lentil to be used as a reference crop for $N_2$ fixation measurement by the natural abundance $\delta^{15}N$ method, as described in Bremer and van Kessel (1990). In the N fertilizer study, CDC Sedley was grown with four rates of 0, 15, 30 and 60 kg N fertilizer $ha^{-1}$ with three replications in both SN and LN. Urea, as the N source, was banded at seeding time.

Seeding, crop management, sampling and recorded variables were identical for both cultivar and N fertilizer studies, except for the $N_2$ fixation measurements in 2008 and fungicide application in all three years, which were limited to the cultivar study only. Lentil was seeded in both locations (SN and LN) with 5.6 kg $ha^{-1}$ granular rhizobia in barley stubble on 5 May 2006, 30 April 2007 and 29 April 2008. Plots were 10.7 m long and 2.5 m wide (27 $m^2$ area) with a row space of 0.20 m. All plots received 58 kg $ha^{-1}$ of seed-placed mono-ammonium phosphate (11-52-0). In 2006 only, 93 kg $ha^{-1}$ potassium sulfate ($K_2SO_4$) was applied to all plots. Amounts of N in each N treatment were corrected for available N from an additional application of mono-ammonium phosphate.

In the first year of study, the herbicides ethalfluralin and glyphosate were applied in September and October 2005, and glyphosate was repeated in May 2006. In the other two years, the pre-seeding herbicide application was limited to glyphosate in September and May of 2007 and 2008. Lentil was sprayed in June with the herbicide sethoxydim in all three years. Weeds not controlled by herbicides were removed three times throughout the season by hand. Plots were sprayed with the fungicide pyraclostrobin in late June or early July (in cultivar study only) in the three years of study.

Plant density was counted three weeks after lentil emergence in three randomly selected rows of each plot, 1 m length each. Lentil was sampled for the aboveground biomass (DW) and N content at three growth stages in both experiments. Four rows, 0.25 m in length were sampled from each plot at full-flower (at least 80% of plants per plots flowered), mid-pod (80% of plants in each plot had >1 pod) and maturity (80% plants per plot turned yellow). Samples were oven dried at 60°C for 24 hrs, weighed and ground. A sub-sample was taken and its N concentration was determined by combustion method, using a Leco carbon-nitrogen determinator (LECO CNS 2000, St. Joseph, MI, USA). In 2008, barley, grown in the cultivar study, was sampled, weighed and ground. A sub-sample from ground material of both lentil and barely was finely ground in a ball mill for 24 hrs. A small fraction of ~1 mg (lentil) to ~3 mg (barley) was encapsulated to be analyzed for N isotope composition on a 20-20 Mass Spectrometer interfaced with an ANCA-GSL sample converter (Europa Scientific, Crewe, UK) according to Bremer and van Kessel (1990). Yield, harvest index (HI) and 1000 kernel weight (KWT) were recorded after applying glyphosate (2006) or diquat (2007 and 2008) as desiccants.

**Data analysis**

Data were analyzed as a combined analysis of variance, with cultivars (or N fertilizer rates) as treatments, NT treatments as location, and year as replication for the location, as explained in Cochran and Cox (1992). Effect of the treatments and locations were tested by the pooled error, calculated by the entire data. This model produced identical output as a split-plot design model which main plots were factorial combinations of tillage duration (LN or SN) and year, and sub-plots were cultivars (in the cultivar study) or N treatments (in the N study). This first model was analyzed in Proc GLM of SAS, version 9.2 (SAS Institute, Cary, NC, 2008). Year and tillage as well as their interactions with other variables were considered as fixed effects. Again, results of this model were similar to a model analyzed in Proc Mixed with year as a random term, or when data were considered as a repeated measure without replication as described in Doncaster and Davey, (2007). Mean of the cultivars or N fertilizer treatments, NT systems and years were separated by LSD, $P<0.05$.

## 6.4 Results

### 6.4.1 Weather conditions

Lentil received a total of 135, 205 and 214 mm rain during the growing seasons (May –August) of 2006, 2007 and 2008, respectively, which corresponds to 11, 55 and 63% of the rainfall occurring in July to August in 2006, 2007 and 2008, respectively. Total rainfall during seed filling and maturity (July and August) in 2007 and 2008 was greater than in 2006, when lentil experienced a terminal drought. In addition, average monthly minimum and maximum temperature varied among the years. The beginning of the growing season (May) in 2006 and 2007 was warmer than the same period in 2008 where its minimum temperatures in May remained around freezing point. Minimum temperature reached below 0 °C in 1, 18 and 22 days during May in 2006, 2007 and 2008, respectively. A total of 119, 62 and 28 heat units ($T_b$ =5°C) by the end of May, and 442, 326 and 273 by the end of June, were accumulated in 2006, 2007 and 2008, respectively (Fig. 6.1).

Late season temperature (August) was noticeably cooler in 2007 than in 2006 and 2008. By maturity, lentil received 1195, 1077 and 979 heat units ($T_b$= 5ºC) in 2006, 2007 and 2008, respectively. These amounts were above (in 2006), equal (in 2007) and below (in 2008) the recommended heat units for lentil maturity in the northern Great Plains (Miller et al., 2002; Gan et al., 2005). The recommended heat units of 414 by flowering (Gan et al., 2005) were received 59, 65 and 75 days after seeding in 2006, 2007 and 2008, respectively.

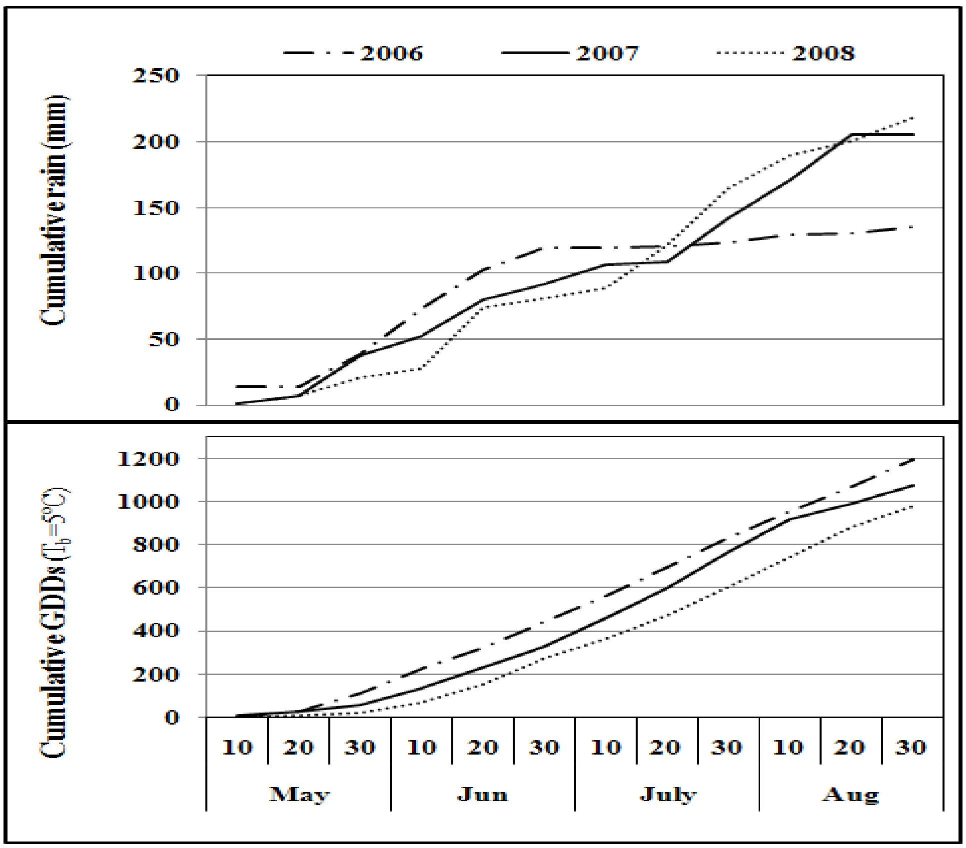


Figure 6.1 Cumulative rainfall (a) and heat (b) during the growing seasons of 2006 to 2008. Data from Environment Canada for the Indian Head CDA station

### 6.4.2 Cultivars performances

From the combined ANOVA, NT duration did not impact any trait in the cultivar study, except the above ground biomass (DW) at maturity (Table 6.2). Interactions of tillage by cultivar, tillage by year and tillage by year by cultivar was observed for some variables. The observed year by length of NT interactions suggests that the response of lentil cultivars to length of NT duration was affected by environmental factors experienced in a given year.

Table 6.2 ANOVA results indicating effects of NT duration (tillage), cultivars and their interactions with year for key measurements on five cultivars of lentil, 2006-2008

| Effect | DF | Yield g $m^{-2}$ | HI % | Seed N % | KWT g 1000 $seed^{-1}$ | Plant DW g DW $m^{-2}$ | Plant N g N $m^{-2}$ |
|---|---|---|---|---|---|---|---|
| Year (Y) | **2** | ** | ** | ns | ns | ** | ** |
| Tillage (T) | **1** | ns | ns | ns | ns | * | ns |
| Cultivar (C) | **4** | * | ** | ** | ** | ** | * |
| T × C | **4** | * | ** | ns | * | ns | ns |
| Y × T | **2** | ns | * | ns | ns | ns | * |
| Y × C | **8** | ns | ** | * | ** | ns | ns |
| Y × T × C | **8** | ns | ** | ns | * | ns | ns |

*, ** and ns indicate significant (P<0.05), highly significant (P<0.01) and not significant differences between tillage, cultivars and their interactions

Averaged for the three growing seasons (years), seed yield was maximized in 2006 (230 g $m^{-2}$) followed by 2007 (214 g $m^{-2}$) and then in 2008 (175 g $m^{-2}$). Patterns of DW and N content among the three years of study were slightly different from the yield pattern. Total DW at maturity in 2006 and 2008 (70 g DW $m^{-2}$) was similarly smaller than DW in 2007 (94 g DW $m^{-2}$). Total plant N content at physiological maturity was 5.3, 6.2 and 7.5 g N $m^{-2}$ in 2006, 2007 and 2008, respectively (Table 6.3). Lentil in 2006 had the highest HI (43%) and in 2008, the least (40%).

Variability of lentil yield between SN and LN in 2006 was independent of HI, because HI did not differ by tillage duration. Instead, the greater yield of lentil in SN compared to LN was linked to higher plant DW and plant N in SN than in LN (Table 6.3). The difference of SN and LN for average yield was not observed in the succeeding years; however, two cultivars produced more yield in SN than in LN in 2007, because of greater HI. In the last year of study (2008), seed yield and DW at maturity were identical in SN and LN, but total plant N content in LN was 1.5 g $m^{-2}$ greater (but not significant) than in SN, likely because of late season rainfall leading to higher N mineralization in LN than in SN. In 2007, and particularly in 2008, lentil was more indeterminate and effects of length of no-till were not apparent.

Table 6.3 Total lentil DW, N content, at flowering (F), mid-pod (P) and physiological maturity (M), and Plant N concentration, HI and yield of lentil at maturity in short-term (SN) and long-term (LN) no-tillage systems for 2006, 2007 and 2008. Mean of five cultivars and three replications.

| Year | Tillage | DW ($g\ m^{-2}$) | | | N content ($g\ m^{-2}$) | | | Plant N (%) | HI (%) | Yield ($g\ m^{-2}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | P | M | F | P | M | Maturity | | |
| **2006** | SN | $38^{c}$ | $142^{bc}$ | $398^{b}$ | $1.5^{bc}$ | $5.1^{ab}$ | $10.6^{c}$ | $2.7^{ab}$ | $42^{abc}$ | $264^{a}$ |
| | LN | $39^{c}$ | $121^{c}$ | $307^{c}$ | $1.4^{c}$ | $3.9^{b}$ | $7.3^{d}$ | $2.4^{b}$ | $45^{a}$ | $195^{bc}$ |
| **2007** | SN | $81^{ab}$ | $188^{a}$ | $485^{ab}$ | $3.9^{a}$ | $5.5^{ab}$ | $12.3^{bc}$ | $2.4^{b}$ | $40^{bcd}$ | $224^{b}$ |
| | LN | $91^{a}$ | $191^{a}$ | $432^{b}$ | $3.5^{ab}$ | $5.6^{a}$ | $12.9^{b}$ | $3.0^{a}$ | $39^{cd}$ | $204^{abc}$ |
| **2008** | SN | $46^{c}$ | $139^{bc}$ | $532^{a}$ | $1.5^{bc}$ | $4.2^{ab}$ | $15.0^{ab}$ | $2.8^{a}$ | $43^{ab}$ | $172^{c}$ |
| | LN | $58^{bc}$ | $167^{ab}$ | $536^{a}$ | $2.2^{abc}$ | $5.3^{ab}$ | $16.5^{a}$ | $3.0^{a}$ | $38^{d}$ | $178^{bc}$ |

Means followed by the same letter within a year × tillage combination are not significant at $P<0.05$

Lentil N concentration ranged 2.9 to 4.1% across all three growth stages and tillage systems and occasionally varied by NT duration. In 2006, lentil had greater N concentration in SN (3.6%) than in LN (3.2%) at mid-pod. Although N concentration is typically diluted in larger biomass, the greater N content ($g\ m^{-2}$) in SN than in LN in 2006 was also linked to greater plant N concentration. The simultaneous high DW and N concentration in 2006 SN implied that SN conditions were suitable for N uptake and $N_2$ fixation. Restricted growth and yield of 2006 LN plants were therefore associated with lower plant N concentration, due to less uptake or fixed N, in LN in that year. In contrast to the first year, lentil had greater N concentration in LN than in SN at both mid-pod and maturity in 2007 (Table 6.3).

The nitrogen supply to lentil was considered in detail in 2008, when $N_2$ fixation was measured. Lentil accumulated N as the season progressed, with the greatest amount (about two thirds of the total N) between mid-pod and maturity (Table 6.4). In 2008 the weather lengthened the reproductive duration between these two stages due to cool conditions, allowing plants to fix or uptake more N. Lentil in LN tended to have greater total N than in SN at each of flowering, mid-pod and at physiological maturity. Cool weather delayed maximum growth of lentil after the mid-pod stage in 2007 and 2008.

Table 6.4 The proportion of total N in lentil coming from fixation, %Ndfa, actual amount of N fixed, and N uptake from soil at flowering (F), full-pod (P) and physiological maturity (M) for lentil grown in short-term (SN) and long-term (LN) no-tillage systems in 2008. Mean of five cultivars and three replications.

| Tillage | **%*Ndfa*** % | | | **Fixed $N_2$†** g $m^{-2}$ | | | **N uptake††** g $m^{-2}$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | **F** | **P** | **M** | **F** | **P** | **M** | **F** | **P** | **M** |
| **SN** | $64^a$ | $84^a$ | $91^a$ | $0.96^a$ | $3.4^a$ | $13.4^a$ | $0.6^a$ | $0.7^b$ | $1.3^b$ |
| **LN** | $52^b$ | $65^b$ | $81^b$ | $1.0^a$ | $3.3^a$ | $13.3^a$ | $1.0^a$ | $1.8^a$ | $3.1^a$ |

Means followed by the same letter within columns are not significant at P<0.05
† Total plant N derived from the atmosphere (g N $m^{-2}$)
††Total plant N uptake from soil (g $m^{-2}$), calculated by subtracting the fixed N from the total plant N content

The proportion of plant N derived from $N_2$ fixation (%*Ndfa*) by lentil cultivars in 2008 was greater in SN than in LN for all sampling times (Table 6.4). Lentil in SN relied more on $N_2$ fixation for its total N requirements than lentil in LN. Differences in lentil %*Ndfa* due to length of no-till was apparent early in the season with greater %*Ndfa* in SN than in LN at flowering, a difference which continued until maturity (Table 6.4). Average %*Ndfa* over cultivars and length of no-till at flowering, mid-pod and maturity were 56, 74 and 80%, respectively, demonstrating that lentil continued fixing N after mid-pod.

Despite differences in %*Ndfa* by the NT systems, total fixed N (%*Ndfa* × total plant N) from both no-till systems was the same for every growth stage (Table 6.4). Both NT systems had the same proportion of N from $N_2$ fixation in terms of contribution to total N, and the LN system could add additional N to plants at mid-pod and by maturity through soil N mineralization. The amount of N taken up by the plant from the soil (total plant N - total fixed $N_2$) was numerically greater for LN than for SN lentil by mid-pod and also at maturity. In 2008, lentil in LN had 1.5 g $m^{-2}$ greater N than lentil in SN, implying that long-term NT could supply more N than short-term in presence of continuous late-season rainfall.

Correlations over the combined data demonstrated a strong ($r=0.97^{**}$, N=60) association between DW and total N (g $m^{-2}$), indicating that more available N increased the growth. However, plant DW was moderately and negatively correlated with yield (r= - 0.40), due to a moderate decline in HI (r= - 0.41 for total N and HI). Overall results, larger plants had more N content (g $m^{-2}$), lower HI and lower yield. Among the three years, HI was negatively and weakly correlated with yield in 2006 ($r= -0.47^{ns}$), but positively correlated with yield in 2007 (r=0.84*) and 2008 (r=0.5*). The 2007 and 2008 relationships suggested that lentil was more vegetative and inefficient in these years and as HI increased, so did yield.

Looking at the main effect of length of no-till, seed yield was similar for both LN and SN as was HI, seed N concentration and total plant N. Lentil plants had greater DW and N concentration in SN than in LN (Table 6.5). Lentil from SN grew one node more than lentil in LN. However, lentil cultivars do not necessarily grow and yield similarly to each other. The ranking of other cultivar yields remained similar regardless of the length in no-till. The highest yielding cultivars were two early maturity groups, CDC Milestone and CDC Robin, with one medium maturity cultivar CDC Vantage. CDC Redcap and CDC Sedley had lower yields.

The two medium maturing cultivars, CDC Sedley and CDC Vantage produced more biomass, followed by the early maturity cultivar CDC Robin. The cultivars with the greater DW and HI values (CDC Milestone and CDC Robin) were well adapted to the varied conditions of the experiment. But CDC Sedley and CDC Vantage had the lower harvest index values, reflecting more vegetative biomass compared to seed yield. The high yield in CDC Vantage was driven by its large DW, not by efficient partitioning to yield, whereas CDC Milestone had superior yield partitioning (by a higher HI) for its lower biomass (Table 6.5).

Table 6.5 Mean separation for the main effect of no-tillage system, short-term (SN) and long-term (LN), cultivar and the tillage by cultivar interaction. Data average over three years and three replications.

| | **Yield** $g\ m^{-2}$ | **HI** % | **Seed N** % | **Density** plant $plot^{-1}$ | **Biomass** $g\ m^{-2}$ | **Total N** $g\ m^{-2}$ | **Plant N** % |
|---|---|---|---|---|---|---|---|
| **No-till duration** | | | | | | | |
| Long-term NT | $193^{a}$ | $44^{a}$ | $4.17^{a}$ | $145^{a}$ | $423^{b}$ | $12.3^{a}$ | $2.8^{a}$ |
| Short-term NT | $219^{a}$ | $44^{a}$ | $4.16^{a}$ | $142^{a}$ | $468^{a}$ | $12.6^{a}$ | $2.6^{b}$ |
| **Year** | | | | | | | |
| 2006 | $230^{a}$ | $45^{ab}$ | $4.12^{b}$ | $122^{c}$ | $353^{c}$ | $9.0^{c}$ | $2.5^{c}$ |
| 2007 | $215^{a}$ | $47^{a}$ | $4.20^{a}$ | $170^{a}$ | $452^{b}$ | $12.6^{b}$ | $2.7^{b}$ |
| 2008 | $175^{b}$ | $40^{b}$ | $4.18^{ab}$ | $140^{b}$ | $534^{a}$ | $15.7^{a}$ | $2.9^{a}$ |
| **Cultivars** | | | | | | | |
| CDC Milestone | $225^{a}$ | $50^{a}$ | $4.03^{b}$ | $151^{ab}$ | $418^{c}$ | $11.6^{a}$ | $2.7^{b}$ |
| CDC Redcap | $188^{b}$ | $45^{ab}$ | $4.32^{a}$ | $141^{bc}$ | $416^{c}$ | $12.4^{a}$ | $2.9^{a}$ |
| CDC Robin | $222^{a}$ | $46^{ab}$ | $4.37^{a}$ | $164^{a}$ | $438^{bc}$ | $13.0^{a}$ | $2.9^{ab}$ |
| CDC Sedley | $191^{b}$ | $39^{b}$ | $4.06^{b}$ | $127^{c}$ | $473^{ab}$ | $12.1^{a}$ | $2.5^{c}$ |
| CDC Vantage | $209^{b}$ | $40^{b}$ | $4.09^{b}$ | $135^{c}$ | $480^{a}$ | $13.0^{a}$ | $2.7^{bc}$ |

Means followed by the same letter for a group within a column (tillage, year, cultivar) are not significant at P<0.05

Although total plant N content at maturity varied from 6.8 to 7.5 g $m^{-2}$, no significant difference was evident between any cultivars (Table 6.5). Overall, node differences ranged from 21 to 22 after rounding the values, but were only different between CDC Vantage and CDC Redcap, implying that CDC Vantage was the largest cultivar with 22 nodes on average across the study, and CDC Redcap was at least one node shorter and had lower DW.

In addition to difference within cultivar performance, the lentil cultivars responded differently to tillage duration. Cultivars CDC Robin had greater yield in SN than in LN, when yield of each cultivar was averaged over the three years (Fig. 6.2). Robin HI was larger in SN (45%) than in LN (39%), it also accumulated slightly greater N in SN (171 g $m^{-2}$) than in LN (147 g $m^{-2}$) by maturity. The ranking of cultivar seed N concentration and DW remained similar for LN and SN. Total plant N contents in SN contained the greatest and least values, compared to midrange values seen in LN plants. Three cultivars had 22 or more nodes in SN, but usually only 21 nodes in LN. Comparison of lentil performance within each year showed that cultivars had greater DW in SN than in LN in 2006 and tended to be greater also in 2007 (except CDC Redcap in 2007).

Comparisons of different cultivars in 2006 and 2007 suggested that HI was effective for yield maintenance under wet conditions of 2007, compared to the fairly dry late-season of 2006. For example, maximized yield in CDC Milestone and CDC Robin in SN than in LN was associated with their higher HI under cool-wet conditions of 2007. Similarly, in the cool-moist conditions of 2008, LN lowered HI of all cultivars except CDC Milestone, which had a constant HI (about 0.47). For the observed cool-wet conditions from mid-pod to physiological maturity in 2007 and 2008, lower HI was evident in the strongly indeterminate and the later maturing cultivars, CDC Sedley and CDC Vantage. If the timing of water and nitrogen supply, driven by rainfall, temperature and length of no-till, coincide with the late stages of flowering, the more indeterminate or later maturing cultivars would show greater vegetative DW but HI would be lowered compared to early maturing cultivars.

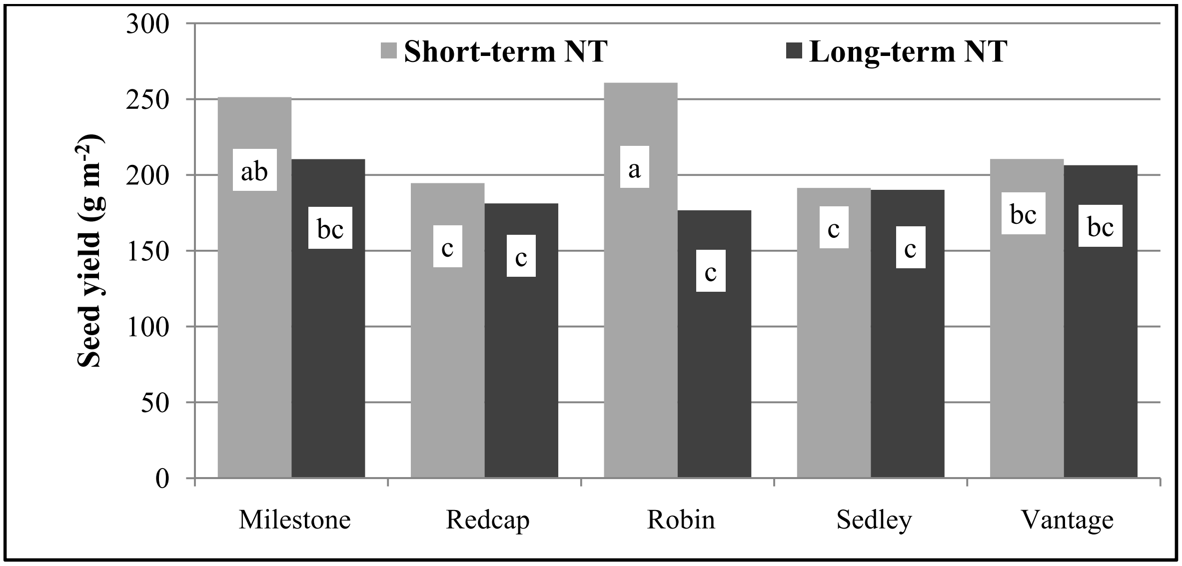


Figure 6.2 Yield of lentil cultivars, averaged over three years, in short and long-term NT. Bars with the same letter are not significantly different ( P<0.05)

### 6.4.3 Nitrogen fertilizer effect

Tillage duration and N fertilizer treatment did not affect average of DW, plant N content and yield of lentil, but CDC Sedley responded to the varied weather conditions in three years of study. Seed N concentration was the only trait was influenced by tillage and N treatment. Interactions of tillage by year were observed for yield and total plant N content at maturity. Similar to the cultivar study, CDC Sedley produced more yield in SN than in LN in 2006 only (Table 6.6). The greater yield of CDC Sedley in SN than in LN in 2006 came from plots receiving less than 60 kg N ha$^{-1}$. In this year, lentil in SN produced 211, 232, 240 and 229 g seed m$^{-2}$ in response to 0, 15, 30 and 60 kg N ha$^{-1}$. In the counterpart plots in LN, Sedley yielded 151, 182, 170 and 210 g seed m$^{-2}$ in response to 0, 15, 30 and 60 kg N ha$^{-1}$, respectively (Fig. 6.3). However, due to variability among lentil plots and lack of sufficient replications, the yield differences were only significant for 0 and 30 kg N ha$^{-1}$ treatments. The lower yield of CDC Sedley in LN compared to SN in 2006 was removed by applying 60 kg N ha$^{-1}$, which suggested that yield of this cultivar was inhibited by N shortage in the LN.

Table 6.6 Significant effects of no tillage duration (tillage), N fertilizer and their interactions with year for key measurements on lentil, cultivar CDC Sedley, 2006-2008.

| | DF | Yield g m$^{-2}$ | HI % | Seed N % | Density plant m$^{-2}$ | Biomass g m$^{-2}$ | Total N g m$^{-2}$ | Plant N % |
|---|---|---|---|---|---|---|---|---|
| **Year (Y)** | 2 | ** | ** | ns | ** | ** | ** | ** |
| **Tillage (T)** | 1 | ns | ns | ** | ns | ns | ns | ns |
| **Fertilizer (N)** | 3 | ns | ns | * | ns | ns | ns | ns |
| **T*Y** | 2 | ** | ns | ns | * | ns | * | ns |
| **T*Y (R )** | 10 | ns | ns | ** | ns | ns | ns | ns |
| **N*Y** | 6 | ns | ns | ** | * | ns | ns | ns |
| **T*N** | 3 | ns | ns | ** | ns | ns | ns | ns |

*, ** and ns indicate significant (P<0.05), highly significant (P<0.01) and not significant differences between tillage, cultivars and their interactions

Similar to the cultivar study, CDC Sedley produced more yield in SN than in LN in 2006 only. The greater yield of CDC Sedley in SN than in LN in 2006 came from plots receiving less than 60 kg N ha$^{-1}$. In this year, lentil in SN produced 211, 232, 240 and 229 g seed m$^{-2}$ in response to 0, 15, 30 and 60 kg N ha$^{-1}$. In the counterpart plots in LN, Sedley yielded 151, 182, 170 and 210 g seed m$^{-2}$ in response to 0, 15, 30 and 60 kg N ha$^{-1}$, respectively (Fig. 6.3). However, due to variability among lentil plots and lack of sufficient replications, the yield differences were only significant for 0 and 30 kg N ha$^{-1}$ treatments. The smaller yield of CDC Sedley in LN than in SN in 2006, which disappeared by applying 60 kg N ha$^{-1}$, may suggest that yield of this cultivar was inhibited by N shortage in the LN.

In agreement with the cultivar study, CDC Sedley produced more yield in LN than in SN in 2008. In 2008, when average yield was smaller than both 2006 and 2007, CDC Sedley produced 52, 43, 41 and 60 g more seed $m^{-2}$ in LN than in SN in response to 0, 15, 30 and 60 kg N $ha^{-1}$, respectively. But, only the difference in the control (0 kg N $ha^{-1}$) was impacted by the NT duration (Fig. 6.3). In 2008, when wet-cool season reduced $N_2$ fixation, more available N in LN than in SN may increase the yield of CDC Sedley.

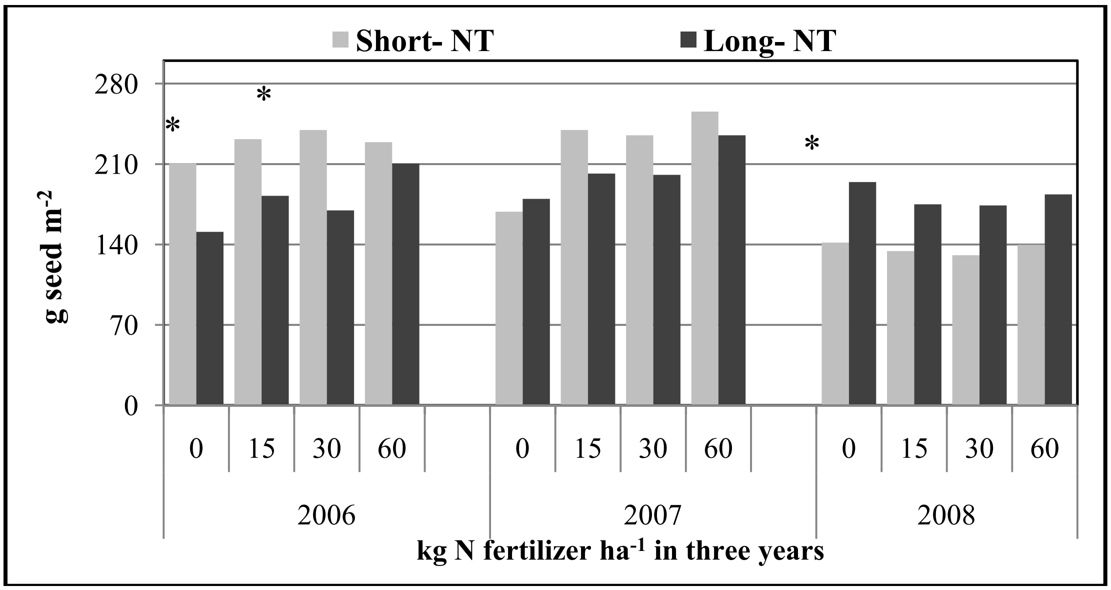


Figure 6.3 Average seed yield of CDC Sedley in long and short term NT and in response to four N fertilizer rates during three years of study. Yield in columns with * is significantly different between LN and SN for the given N fertilizer treatment.

Averages HI, seed N concentration, plant DW, plant N content and plant N concentration of CDC Sedley over the three years of study were identical in SN and LN (Table 6.7). However, comparison for the year effect showed that CDC Sedley accumulated more than twice DW and N by maturity in 2007 as it did in 2006. Interestingly, a greater biomass in 2007 and 2008, compared to 2006, was followed by about 0.6% greater plant N concentration. However analysis of variance failed to demonstrate significant differences in plant N concentration among the three years (Table 6.6, 6.7).

Table 6.7 Yield, HI, seed N concentration, plant density, and total plant DW, N content and plant N concentration at maturity, for cultivar CDC Sedley grown in short-term and long-term NT (averaged over year, N treatments and replications), for 2006, 2007 and 2008 (averaged over tillage treatments, N treatments and replications) and for four N treatments (averaged over tillage treatments, years and replications).

| | Yield g $m^{-2}$ | HI % | Seed N % | Density plant $plot^{-1}$ | Biomass g $m^{-2}$ | Total N g $m^{-2}$ | Plant N % |
|---|---|---|---|---|---|---|---|
| **No-till duration** | | | | | | | |
| Long-term NT | $188^{a}$ | $41^{a}$ | $4.2^{a}$ | $130^{a}$ | $365^{a}$ | $8.5^{a}$ | $2.3^{a}$ |
| Short-term NT | $203^{a}$ | $40^{a}$ | $4.0^{b}$ | $130^{a}$ | $386^{a}$ | $9.1^{a}$ | $2.3^{a}$ |
| **Year** | | | | | | | |
| 2006 | $203^{a}$ | $33^{a}$ | $4.1^{b}$ | $118^{b}$ | $269^{c}$ | $5.12^{c}$ | $1.9^{b}$ |
| 2007 | $225^{a}$ | $53^{a}$ | $4.1^{b}$ | $170^{a}$ | $508^{a}$ | $12.7^{a}$ | $2.5^{a}$ |
| 2008 | $159^{b}$ | $34^{a}$ | $4.2^{a}$ | $101^{c}$ | $350^{b}$ | $8.6^{b}$ | $2.5^{a}$ |
| **N Treatments** | | | | | | | |
| 0 kg N $ha^{-1}$ | $183^{a}$ | $39^{a}$ | $4.2^{a}$ | $127^{a}$ | $383^{a}$ | $8.7^{a}$ | $2.2^{a}$ |
| 15 kg N ha | $194^{a}$ | $40^{a}$ | $4.1^{ab}$ | $134^{a}$ | $356^{a}$ | $8.2^{a}$ | $2.3^{a}$ |
| 30 kg N ha | $197^{a}$ | $42^{a}$ | $4.1^{ab}$ | $124^{a}$ | $380^{a}$ | $9.2^{a}$ | $2.4^{a}$ |
| 60 kg N ha | $209^{a}$ | $40^{a}$ | $4.0^{b}$ | $134^{a}$ | $385^{a}$ | $9.0^{a}$ | $2.3^{a}$ |

Means followed by the same letter are not significant for tillage, year and N treatment effects ($P<0.05$).

The greater yield of CDC Sedley in SN than in LN in 2006 was independent of HI and seemed to be associated with slightly greater DW and N content of lentil in SN than in LN (Fig 6.4). In 2007, slightly greater yield (21 g seed $m^{-2}$) of CDC Sedley in SN than in LN was associated with slightly greater DW, N content (Fig. 6.4) and HI (about 3%) of lentil in SN than in LN. In 2008, in agreement with the effect of NT duration on seed yield, CDC Sedley accumulated more DW and N in LN than in SN; however, the differences of DW were not significant (Fig. 6.4). Plant density was different among years, and on average, 118, 170 and 101 plants $m^{-2}$ was counted for 2006, 2007 and 2008, respectively (Table 6.7). The varied plant density, which was not observed among the N fertilizer treatments or between NT systems, was not considered in data analysis. Noticeably greater plant density in 2007 than in two other years may be associated with its greater biomass and N content.

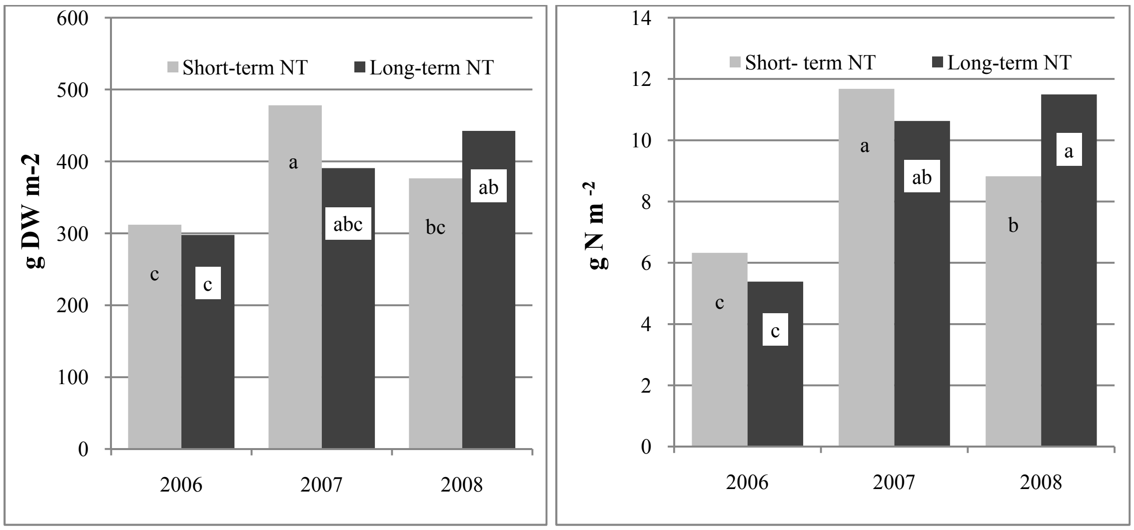


Figure 6.4 Average DW (left) and N content (right) of CDC Sedley in SN and LN during three years of study. Columns contained similar letter are not significant.

Interaction between N fertilizer rates and NT duration was observed. In 2007, lentil had 34 g less DW $m^{-2}$ in SN than in LN without N fertilizer (0 kg N), but 37, 83 and 22 more DW $m^{-2}$ in SN than in LN with 15, 30 and 60 kg N $ha^{-1}$, respectively. A similar pattern was observed in 2008, when CDC Sedley had 27 g $m^{-2}$ less DW in SN than in LN without N fertilizer (0 kg N treatment), but this variation reversed and Sedley had 29 g $m^{-2}$ more DW in SN than in LN by 30 kg N $ha^{-1}$ (data not presented). The improved yield of CDC Sedley in SN with additional N fertilizer suggested that varied yield of lentil between SN and LN was associated with N availability.

In the last year, when lentil yield was lowest in cool-wet conditions, DW was greater in LN than in SN, regardless of the N fertilizer treatments. Interestingly, total N content, which always followed the same pattern as DW, had a different trend in this year. In the 60 kg N $ha^{-1}$ treatment, total lentil N in LN was 4.4 g $m^{-2}$ smaller in SN, whereas DW in LN was 60 g $m^{-2}$ greater than in SN. This variation suggested that excessive moisture stimulated vegetative growth, but the biomass was depleted in N.

Biomass and total N of CDC Sedley at flowering and mid-pod also responded to the N fertilizer treatments, NT durations and weather effects. At flowering, CDC Sedley had greater DW and N content in SN than in LN across the N fertilizer treatments in both 2006 and 2007, with the greatest differences in the 60 kg N $ha^{-1}$ treatment. Plant DW and N prior to seed filling in 2008 were different from the two previous years, and CDC Sedley had greater N and DW in LN than in SN, regardless of N fertility treatments. Fig. 6.5 shows overall trends in DW, N content and N concentration of lentil plants at three stages of growth.

Average seed N concentration over the three years was greater in LN than in SN (Table 6.7). Seed N concentration was greater in LN than in SN across the N fertilizer treatments in 2008 and among some treatments in 2006. In contrast, seed N concentration was greater in SN than in LN in 2007. Variation in seed N concentration among treatments, NT systems and year was explained by varied yield and N availability. Greater seed yield normally dilutes the concentration of N in seed.

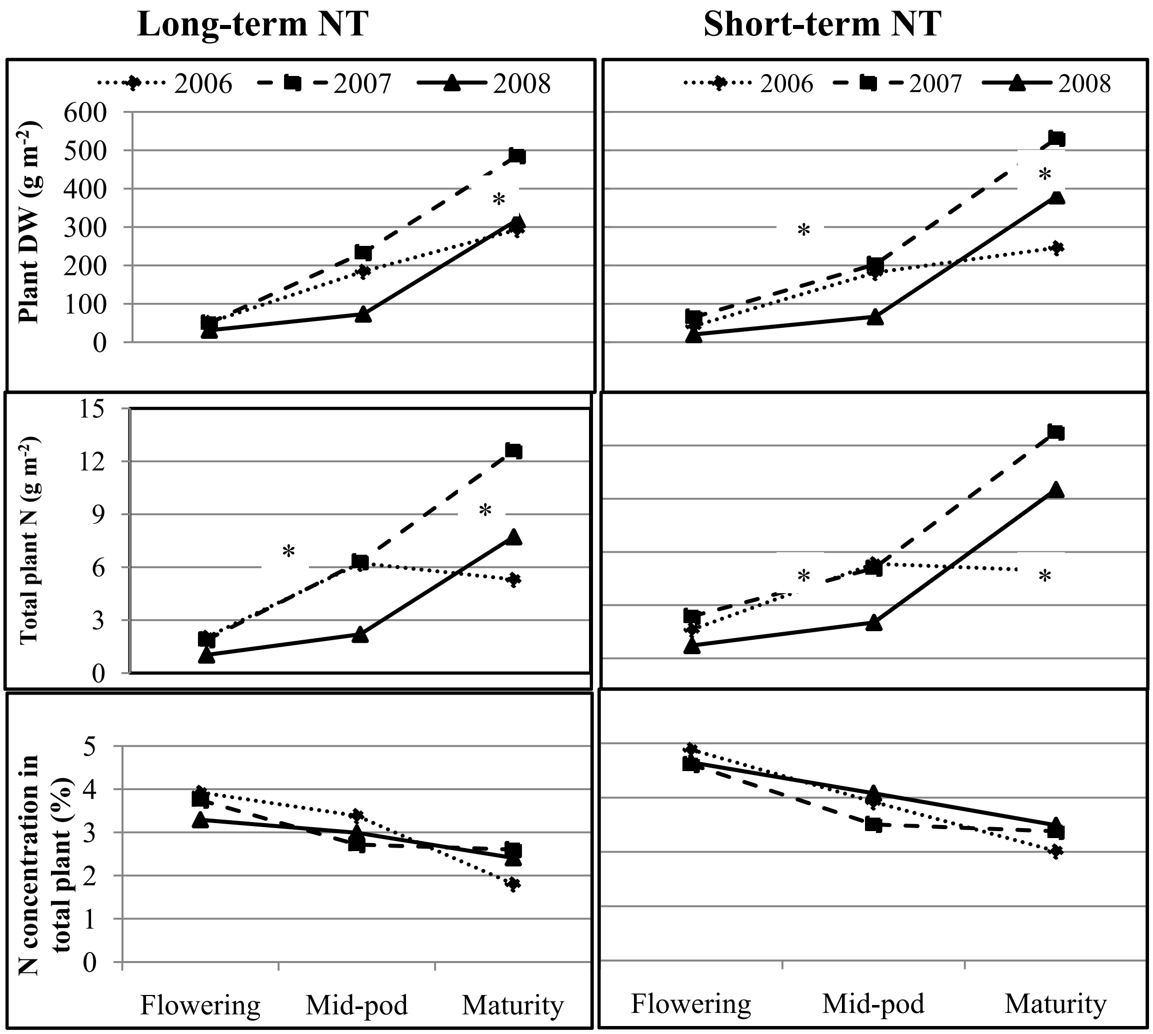


Figure 6.5 Plant DW (top), total plant N (middle) and plant N concentration (bottom) of lentil at three stages of flowering, mid-pod and maturity in long and short –term NT. Significant effect of year for each growth stage is indicated by *.

## 6.5 Discussion

### 6.5.1 Environment effects

Lentil yield was maximized in 2006, when total rainfall was 57% of the rainfall in the other two years. Sufficient early to mid season rainfall, warm temperature and the mild terminal drought seemed to be suitable for lentil yield in 2006. These results are in agreement with findings by McKenzie and Hill (1990) and Whitehead et al. (2000) who measured higher yield in lentil subjected to less water than lentil grown in wet conditions. Comparison of yield in 2006 and 2007 suggested that a short period of warm temperature in late July and early August in 2007 could lower the undesired effects of excessive moisture, as lentil produced almost similar yield in wet 2007 as it did under dry conditions of 2006. The unexpected lowered yield of lentil in 2008 was associated with insufficient heat accumulation, which did not provide require GDD for lentil maturity (Miller et al., 2002; Gan et al., 2005).

In the cultivar study, 50, 40 and 60% of the final DW was accumulated after mid-pod in 2006, 2007 and 2008, respectively. From these amounts, 35, 52 and 67% was vegetative biomass and the remaining of 65, 48 and 33% was seed yield in 2006, 2007 and 2008, respectively; indicating that 2008 was the year that produced the most vegetative dry matter by maturity. Whitehead et al. (2000) measured more biomass but smaller HI and seed yield in lentil grown in the wet-cold environment of Pullman, WA (1997) and Reading, UK (1997) compared to the warm-dry conditions at Pullman in 1996. A negative effect of cool temperature on seed N concentration during seed-filling in pea (*Pisum sativum* L.) was observed by Larmure et al., (2005).

Despite HI being more stable than seed yield, HI was greater in 2006 than in 2007 and 2008 (Table 6.4). These data provide a convincing case that excessive mid to late-season growth lowers HI and yield in cooler or wetter years for indeterminate crops, and particularly for the later-maturing lentil cultivars. Variability of legume HI in different environments is associated with N availability (Sinclair, 1998), indeterminate growth (Wallace et al., 1990) and lower biomass by leaf loss (Hay, 1995). The lower association of lentil yield with HI in 2006 than in 2007 is opposed to variability of yield and biomass among the years, as cool conditions of 2007 could lower partitioning of DW and N to seeds (Larmure et al., 2005; Whitehead et al., 2000).

In addition to the weather effects, the plant DW and N prior to seed-filling could have been ameliorated by different plant population densities in each year. As a means of compensating growth, lentil produces more stems and branches at lower densities (Wilson and Teare, 1972). Stand counts among the years in both cultivar study (Table 6.4) and N fertilizer trial (Table 6.7), but this variation was not considered as an effective variable in data analysis, similar to the Gan et al. (2005).

### 6.5.2 Effects of NT duration

Similar to response of lentil to NT duration within the three years of study , response of pea yield to the NT duration in the same location differed by year (Lafond et al., 2008). The authors believed that low yield in LN was associated with late season growth and indeterminacy from excessive soil N supply. In the current study, the varied response of lentil yield to the NT systems in different years may be associated with different patterns of $N_2$ fixation and N accumulation in lentil. In 2006, a warmer early season could increase $N_2$ fixation, whereas late season drought restricted plant growth, root extension and N uptake. Under the circumstance in 2006, inhibited $N_2$ fixation due to more available N in LN (see Table 6.1 and also Schoenau et al., 2008) resulted in reduced growth and yield of lentil in the LN. Considering that maximum growth and N accumulation occurs in lentil from mid-pod to maturity (Table 6.4, Fig. 6.5), post-flowering N availability (or fixation) is directly associated with increased lentil yield (see Fig. 5.1 and 5.2 in Chapter 5 for more details). Late-season N mineralization was limited by terminal drought in 2006; hence, variation of lentil yield in LN and SN appeared.

In 2007, continuous rainfall during plant growth could increase the late-season N uptake; therefore, any inhibition in $N_2$ fixation in LN was compensated by higher N uptake. However, cultivars with greater HI, and possibly more $N_2$ fixation, could increase their yield in SN than in LN in 2007. In this year, CDC Milestone and CDC Robin had greater total N content, N concentration and yield in SN over LN. The greater N, likely from fixation, of these cultivars in SN than in LN together with greater HI (~ 46%) could improve their yield in SN than in LN. Finally, cool temperature and wet conditions of 2008 considerably limited $N_2$ fixation; hence, LN lentil, which had access to greater N, improved its growth and yield.

The only measurement of $N_2$ fixation in 2008 demonstrated the greater $N_2$ fixation of lentil in SN than in LN throughout the entire season (Table 6.5). By assuming a similar fixation pattern for 2006 and 2007, the lowered yield of lentil in LN may be associated with extra nitrate suppressing plant $N_2$ fixation. The inhibiting effect of soil nitrate on nitrogenase activity is well documented in literature (see the detailed review by Luciñski et al., 2002). In contrast to the results of my study, van Kessel and Hartley (2000) concluded that improved moisture availability in NT systems has increased $N_2$ fixation of pulse crops in the American Great Plains. Peoples et al.

(1995) also found that lowered oxidation rates of organic matter and lower nitrate concentration in NT than CT was associated with improved $N_2$ fixation crops under NT systems. As lentil had access to similar water at both locations, the varied N availability between LN and SN (Table 6.1, Lafond et al., 2008; Schoenau et al., 2008) affected $N_2$ fixation and yield of lentil, when late season N uptake was limited by drought.

Interestingly, applying 60 kg N $ha^{-1}$ reduced the variation of DW, N and yield of lentil between SN and LN in the dry year of 2006, when LN lentil was limited by N. Again, the greater yield of CDC Sedley in LN than in SN in 2008 was disappeared by applying 60 kg N fertilizer $ha^{-1}$ (Fig. 6.3), suggesting that SN lentil was limited by N availability in cool-wet conditions of 2008.

Other soil chemical properties could also affect the performance of lentil under two NT systems, especially in 2006. Slower degradation of herbicides or crop injury from ethalfluralin and glyphosate could possibly occur. Ethalfluralin is a registered herbicide for fall application on lentil only, and a risk of lentil injury exists in cold soils; Crop Protection Guide, 2010). Glyphosate is also registered for application prior to or immediately after seeding in lentil. Carry over effects of herbicide do occur in cold soils or high latitudes climates due to incomplete breakdown or desorption of herbicide from clay (Laitinen et al, 2006).

Averaged over three years, CDC Robin produced more yield in SN than in LN and CDC Milestone tended to have the same pattern. Mean separation by year demonstrated that these cultivars, which had greater yield than the other cultivars, produced more yield in SN than in LN in both 2006 and 2007. This variation suggested that in the Black Soil Zone of western Canada, great $N_2$ fixation, in the presence of high soil N, together with stable to high HI in cool-wet weather, are two important elements of achieving high yield in lentil. In contrast, CDC Sedley produced one of the lowest yields among the cultivars, despite its significantly greater DW and N than most other cultivars. The other low yielding cultivar was CDC Redcap, which had reasonable HI (46%), but lowered DW and N, most likely due to low $N_2$ fixation. However, the $N_2$ fixation measurement did not identify any cultivar variability under unusual conditions of 2008.

## 6.6 Conclusion

Overall results rejected the main hypothesis of the study, that prolonged NT could stimulate growth of lentil and reduce the yield. Instead, growth and yield of lentil under wide range of N availability demonstrated that higher yield in lentil was linked to more N uptake or fixation. Although excessive rainfall stimulated vegetative growth of lentil, less N availability or inhibited $N_2$ fixation would not lower the undesired effects of indeterminate growth on yield. Occasionally low yield of cultivar CDC Sedley was independent of excessive N availability. Post-flowering growth and N accumulation was important in yield formation and demonstrated that reduced N availability reduced lentil yield. Greater $N_2$ fixation and efficiency of cultivars for partitioning of carbohydrate and N to seed should therefore be considered in cultivar recommendations for areas similar to the place of study. Cultivars such CDC Milestone and CDC Robin can maintain high $N_2$ fixation under cool temperature and can also dedicate a greater portion of plant biomass to seed.

Varied performance of lentil under two tillage systems and within three years suggested that additional N in LN compared to SN may restrict lentil $N_2$ fixation. However, the inhibited $N_2$

fixation was less apparent, when adequate late-season rainfall extended the duration of N uptake of the LN lentil. Considering the average long-term rainfall of the region compared to the drought conditions of 2006 suggested that lentil yield is less likely to be affected by duration of NT. Also, lentil yield in the Black Soil Zone is more likely to be limited by cool temperature than by excessive moisture. A more detailed experiment on the effect of NT duration on lentil $N_2$ fixation and yield under varied temperature and moisture should be conducted to address this finding.

## 7.0 Effects of N on Reproductive Duration and Yield in Lentil Cultivars

### 7.1 Abstract

Reduced nitrogen (N) uptake and $N_2$ fixation during the reproductive growth may reduce the yield in lentil (*Lens culinaris* Medic.). Five cultivars were grown in two soil media and four N treatments of an inoculated control (NI), and receiving N until flowering (NF), podding (NP) and maturity (NM) in Saskatchewan, Canada. The NF treatment produced the lowest yield, and plants relying on fixation grew larger and yielded more. But NI produced less growth and yield than NP and NM plants, demonstrating that $N_2$ fixation needed improvement for maximal yield. The NP treatment produced the highest yield and more efficiently used N than the later N application in NM. Nitrogen applied at and after mid-pod extended the days to maturity by increasing the reproductive growth period and post-flowering vegetative biomass. Current lentil cultivars require N supply up to at least the mid-pod stage, although the exact stage and timing of N supply need refinement.

### 7.2 Introduction

In favorable conditions, lentil inoculated with rhizobia obtains up to 85% of its N requirements via $N_2$ fixation (van Kessel 1994; Kurdali et al., 1997). However, lentil yield in high-yielding environments may be limited by plant N (Whitehead et al., 2000). Biomass, N accumulation and $N_2$ fixation in lentil were seen to be synchronized under both water available (van Kessel 1994) and water deficient (Kurdali et al., 1997) environments. In Saskatchewan and under irrigated conditions, rate of biomass accumulation during the reproductive growth in lentil cultivar laired was almost as twice (104%) as the rate of N accumulation (52%). Diluted N in a larger biomass along with significantly high seed N demand during the reproductive growth induce chlorophyll breakdown and N (and carbon) remobilization from leaves and stems to seeds and induce leaf senescence and leaf loss (Gutiérrez-Boem et al., 2004).

Plant litter representing leaf loss accounted for 112 to 764 kg litter $ha^{-1}$ from different cultivars of lentil, which were more depleted in N as growing season progressed (Whitehead et al., 2000). In irrigated lentil in Saskatchewan, 22 kg N $ha^{-1}$ (van Kessel 1994) and in dryland lentil in Syria, 53 kg N $ha^{-1}$ (Kurdali et al., 1997) were translocated from above ground biomass to seeds. Remobilized N during reproductive growth can improve yield in dry areas, when growing season is terminated by the drought. However, in the presences of sufficient water, which seed-filling rate may be restricted by cool temperature (Larmure et al., 2005), N remobilization may not be sufficient to maximize the yield.

Under the late-season N deficient conditions, post-flowering N application may increase yield of legume crops via improving leaf photosynthesis, without inhibiting plant $N_2$ fixation (Salvagiotti, F. 2008). Application of N to soybean (*Glycine max* L. Merr) at flower initiation resulted in a greater seed yield, dry matter and plant N content compared to an untreated control and to plants receiving 120 mg N $pot^{-1}$ prior to seeding (Isfan 1991). Late maturing cultivars in this experiment accumulated more N because of their larger biomass. Conversely, in dryland soybean, seed yield did not increase by N application at pod elongation or seed fill stage (Schmitt et al., 2001). Capability of cultivars for $N_2$ fixation, plant duration from seeding to maturity and environmental conditions influence response of plant to the application of N during reproductive growth (Freeborn et al., 2001). By comparing early and late senescing cultivars in soybean, greater acetylene reduction in nodules on cultivars with delayed senescence indicated greater ongoing $N_2$ fixation activity (Abu-Shakra et al., 1978).

In Saskatchewan, lentil yield may decline via lowered harvest index, delayed maturity and frost damage. Hence, lentil cultivation is restricted to soil with low available N or to earlier maturating cultivars. However, insufficient plant N during reproductive growth or hastened maturity may reduce the yield. Despite the extensive studies on the effect of starter N fertilizer on chickpea (*Cicer arietinum*) and lentil (Bremer et al., 1989; Cowell et al., 1989; Gan et al 2009a), possible yield loss of these two indeterminate pulses due to late season N deficiency has not been investigated. We hypothesized that post-flowering N supply will delay leaf senescence and increases days to maturity and yield of lentil compared to an inoculated control.

## 7.3 Materials and Methods

### 7.3.1 Experimental design

Five cultivars of lentil were grown under four N treatments in greenhouse at the University of Saskatchewan (52. 1̊ N and 106. 6̊ W) under an 18/6 hr day/night cycle. The cultivars, currently grown in western Canada, were CDC Greenland, CDC Sedley (large green-seed, late maturity group), CDC Milestone (small green-seeded, early maturity group), and CDC Blaze and CDC Rouleau (small red-cotyledons, early and medium maturity groups). Barley (*Hordeum vulgare*) cv. CDC Dolly was grown as a reference crop for estimating biological $N_2$ fixation using the natural $\delta^{15}N$ abundance method. The inoculated treatment (NI) received 4 mg starter N plant$^{-1}$ two weeks after plant emergence. The three non-inoculated treatments received a total of 34 mg N plant$^{-1}$ from two weeks after emergence until flowering (treatment NF), 84 mg N plant$^{-1}$

until mid-pod (treatment NP) and 144 mg N plant$^{-1}$ until maturity (treatment NM), as in table 7.1. Maximum applied N to lentil by the end of each growth stage was equal to the amount of accumulated N in the paper by van Kessel (1994).

Table 7.1 Accumulated N by lentil as estimated by van Kessel (1994), and the maximum applied N in each treatment in this study

| From Van Kessel (1994) | | | Maximum applied N in the study | |
|---|---|---|---|---|
| Days after planting | Assumed growth stage | Cumulative N (kg ha$^{-1}$) | Treatment | (mg N plant$^{1}$)† |
| 14 | Seedling establishment | -- | **NI** | 4 |
| 41<br>51 | Flowering | 15. 4<br>22. 2 | **NF** | 34 |
| 62<br>71 | Pod formation | 69. 1<br>83. 7 | **NP** | 84 |
| 86<br>118 | Seed filling period | 140.1<br>149. 4 | **NM** | 144 |

†Nitrogen requirement was calculated based on the assumption of 100 plant m$^{-2}$ in the study by van Kessel (1994)

The experiment was conducted in two runs in two different soil media, M1 and M2. Pot media for M1 was autoclaved Sunshine Mix number 4 (N-free peat-perlite-vermiculate mixture, Sungro Horticulture Inc., Ontario, Canada). Pot media for M2 contained an equal mixture of field soil, river sand and the Sunshine Mix. Field soil for M2 was collected from the top 0.30 m of a field with no history of legume crops, located in the brown soil zone of Saskatchewan. The average soil bulk densities were 0.20 and 1.17 Mg m$^{-3}$ in M1 and M2, respectively, after 24 hr oven drying of soil at 60°C. Initial N content in M1 was 48 and 26 µg g$^{-1}$ of $NO_3^-$ and $NH_4^+$, respectively; M2 contained 7 and 29 µg g$^{-1}$ of $NO_3^-$ and $NH_4^+$, respectively.

Both soil media were autoclaved at 120 ºC for 30 min, and 1.5 L cylindrical black pots were filled with either media. Pots were saturated with water, drained for 24 hr, and then reweighed.

This drained saturated weight was referenced as the target pot capacity for watering pots during the growing season. Lentil seeds were surface sterilized in 95% ethanol for 30s, followed by repeated washing in distilled water. Six sterilized seeds of each cultivar were sown at a depth of 30 to 40 mm of pot. Seeds were inoculated with 2 to 3 g $pot^{-1}$ granular commercial rhizobia (Becker Underwood, Saskatoon, SK) in the seeding zone (Kyei-Boahen et al., 2002) for the inoculated treatment. Pots were thinned to three plants $pot^{-1}$ two weeks after emergence at the 5 to 8 leaf stage. Plants received daily amounts of distilled water to bring pot weights back to the target pot capacity during the experiment.

Lentil was seeded on July 22, 2008. The average daily temperature in the greenhouse during the growing season was 25.6 °C for July, 26.6 °C for August, 24.8 °C for September, 21.8 °C for October and 20.4 °C for November. Both N-free and N-included modified Hoagland solutions (Table 7.2) from de Silva et al., (1996) were applied every two weeks and plants continued to receive distilled water to make up the daily target pot capacity with the nutrient solution. Each run (soil media) had four N treatments, five cultivars and three replications that were repeated three times for three destructive sampling at flowering, podding and maturity (total of 180 pots per run). Pods were arranged in a completely randomized design with a factorial combination of cultivars and N treatments for each run.

### 7.3.2 Data collection and sampling

In addition to phenological records of days to flowering and days to maturity, above ground dry matter (DW) was sampled at three stages of flowering (at least one open flower $pot^{-1}$), mid-pod (at least 5 pods $pot^{-1}$), and maturity (70 to 80% of plant organs $pot^{-1}$ turned yellow). Samples were dried at 60°C for at least 24 hr and weighed. Samples were then ground and N concentration was measured by combustion (LECO CNS 2000, St. Joseph, MI, USA). At maturity, dried samples were first hand-threshed and seeds were counted and weighed. Harvest index (HI) was calculated as seed weight divided by total plant DW of the above ground plant material. Seeds were then ground together with the leaves and stems for N measurement. Total plant N content was calculated by multiplying plant DW by the plant N concentration.

Table 7.2 Modified Hoagland solution recipe from de Silva et al. (1996)

| | **Chemical** | **Commercial name** | **Mol. Wt** | **Concentration** | **g $L^{-1}$ (in stock)** |
|---|---|---|---|---|---|
| **Stock #** | **Major Elements** | | | | |
| **1** | $K_2PO_4$ | Potassium Phosphate | 136.09 | 0.2 M | 27.22 |
| **2** | $NH_4NO_3$ | Ammonium nitrate† | 80.04 | 1 M | 80.04 |
| **3** | $MgSO_4(7H_2O)$ | Magnesium Sulfate | 246.47 | 0.4 M | 98.59 |
| **4** | $KC_2H_3O_2$ | Potassium Acetate | 176.2 | 1.0 M | 98.14 |
| **5** | $CaC_4H_6O_4$ | Calcium Acetate | --- | 1.0 M | 176.2 |
| | **Trace elements** | | | | |
| **6** | $H_3BO_3$ | Boric Acid | 61.8 | --- | 0.57 |
| **7** | $MnCl_2(4H_2O)$ | Manganese Chloride | 197.9 | --- | 0.36 |
| **8** | $ZnSO_4(7\ H_2O)$ | Zinc Sulfate | 287.6 | --- | 0.04 |
| **9** | $CuSO_4(5\ H_2O)$ | Copper Sulfate | 249.7 | --- | 0.016 |
| **10** | $H_2MoO_4(H_2O)$ | Molybdic Acid | 162.4 | --- | 0.004 |
| **11** | Fe EDTA | Chelated Iron | 38.2 | 0.5 M | 19.01 |

† Ammonium nitrate was eliminated from the recipe for making the N-free solution

Lentil $N_2$ fixation in the inoculated treatment was measured at physiological maturity using the natural abundance $\delta^{15}N$ isotope method. For this purpose, a fraction of the ground lentil (from inoculated treatment) and barley was ball-milled (Planetary Ball Mill PM100, Retsch Inc., Newtown, PA). Samples were analyzed for isotopic N composition on a 20-20 Mass Spectrometer interfaced with an ANCA-GSL sample converter (Europa Scientific, Crewe, UK).

The percentage of the derived N from atmosphere (*%Ndfa*) in the aboveground biomass was calculated as described in Bremer and van Kessel, 1990.

At harvesting, 20 to 30 g soil $pot^{-1}$ was taken, ground and its available N was extracted by 2M KCL method (Keeney and Nelson,1982), and the residual soil nitrate ($NO_3^-$) and ammonia ($NH_4^+$) were measured by an Autoanalyzer. Changing in plant vegetative biomass (VDW) during seed filling (flowering to physiological maturity) was estimated by the following equation:

$$\boldsymbol{VDW = [Total\ DW\ at\ maturity - seed\ yield] - Total\ DW\ at\ flowering}$$

### 7.3.3 Data analysis

Collected data from both runs of experiments were first pooled together and analyzed, using the Proc Mixed procedure of SAS (SAS Institute Inc. 2008). By considering soil media as a fixed factor, significant differences were observed between the soil media. To avoid the confounding variations of the soil media on the results, data were then analyzed separately for each soil medium.

## 7.4 Results

### 7.4.1 Analysis of variance

Separate analysis of variances for each run of experiment, M1 and M2, showed significant differences among N treatments and cultivars. Interactions of N treatments and cultivars were often significant in M2, but not in M1 (Table 7.3). The significant interactions of N treatments and cultivars in M2 were attributed to the greater $N_2$ fixation, DW, N content and yield of cv. CDC Rouleau compared to other cultivars in the NI treatment.

Table 7.3 ANOVA for growth and yield of lentil at maturity in soil media M1 and M2.

| Effect | DF | DW | N% | Total N Content | Seed Yield | Seed Weight | Harvest Index | Days to Flowering | Days to maturity | Residual Soil N† |
|---|---|---|---|---|---|---|---|---|---|---|
| **M1 media** | | | | | | | | | | |
| N treatment | 3 | ** | ** | ** | ** | * | ns | ns | ** | ** |
| Cultivar | 4 | ns | * | ns | ns | ** | ** | ** | ** | ns |
| N × Cultivar | 12 | ns | ns | ns | ns | ns | * | ns | ns | ns |
| **M2 media** | | | | | | | | | | |
| N treatment | 3 | ** | ** | ** | ** | ns | ** | ns | ** | ** |
| Cultivar | 4 | ** | ** | ** | ** | ** | ** | ** | ** | ns |
| N × Cultivar | 12 | ** | ** | ** | ** | ns | ** | ns | ** | ns |

* ,** and ns indicate differences between nitrogen treatment means, cultivars or the interaction term are significant at $P<0.05$, $P < 0.01$ or not significant, respectively
†Total soil available N, measured after the harvesting at maturity

Seed yield was significantly affected by N treatments. Nitrogen applied until mid-pod (NP) and maturity (NM) resulted in similar and significantly greater seed yield than applying N until flowering (NF). Inoculated lentil (NI) had a lower average yield but it was not significantly different compared to NP and NM yield. The lowest final plant DW, N concentration and total plant N content, and seed yield values in both soil media came from the NF treatment (Table 7.4). Total plant DW and N content significantly increased when more N was given to the plants from flowering to mid-pod (NP) or from flowering to maturity (NM). The synchronized low DW and low N concentration of lentil plants receiving N only up to flowering (NF) indicated that plants were severely N-deficient and performed poorly compared to other treatments.

Similar to the yield response, maximum DW, plant N concentration and plant N content (g N plant$^{-1}$) improved via the post-flowering N application treatments. Maximum DW, N concentration and N content of lentil came from NP and NM treatments, followed by NI and NF treatments. The smaller plant N content in NI compared with NP and NM suggested that $N_2$ fixation in the inoculated lentil could not provide sufficient N to meet plant N requirements. The average DW and N content of the inoculated plants over both soil media were 3.8 g and 116 mg plant$^{-1}$, respectively (Table 7.4).

Supplying N after first flower in NP resulted in 49 and 66% greater DW and N content compared to the application of N until flowering in the NF treatment (averaged over two soils). The higher plant N content of the two later treatments (NP and NM), compared to NF, showed that lentil could efficiently uptake and utilize N from soil during the reproductive phase and N supplied during reproductive growth was essential for growth and yield. However, further application of N after the mid-pod stage did not change the plant DW, N content and seed yield significantly when compared to the NP treatment (Table 7.4). Lentil in NP and NM treatments was not inoculated and could not fix $N_2$, so the similar DW and N content in NM and NP demonstrated that lentil could not effectively use N applied after mid-pod.

By physiological maturity, all cultivars, except CDC Rouleau, had more DW and N content in NI than in NF. CDC Rouleau flowered almost 10 days later than other cultivars, which extended the period of pre-flowering N application in NF as well as the duration of pre-flowering $N_2$ fixation in NI. Response of different cultivars to the later N treatments varied in different soil media. In the first soil media, NM and NP produced larger plants but not necessarily a greater yield over the NI control. In the second soil media, where vegetative growth was restricted by heavier soil, the greater plant N content in NP and NM compared to NI and NF increased seed yield in all cultivars except in CDC Blaze. Unexpectedly, both the plant DW and N content of CDC Blaze and CDC Milestone declined when they received N after mid-pod. This declined of DW and N due to the post-pod N application (NM) could result from salt (toxicity) stress that increased the proportion of lost leaves.

Table 7.4 Plant DW, nitrogen concentration (%), total plant N content, yield and the percentage of N derived from fixation (%Ndfa) of five lentil cultivars in response to four N treatments in soil media M1 and M2. Plants were measured at physiological maturity†.

| Media | N treatment | Cultivars | DW (g plant $^{-1}$) | Plant N % | N Content (mg plant $^{-1}$) | Yield (g plant $^{-1}$) | *%Ndfa* |
|---|---|---|---|---|---|---|---|
| M1 | **NI** (Inoculant No-N) | | $4.3^{b}$ | $2.8^{c}$ | $122^{b}$ | $1.3^{ab}$ | - |
| | **NF** (N until flowering) | | $3.2^{c}$ | $2.2^{d}$ | $70^{c}$ | $1.0^{b}$ | - |
| | **NP** (N until mid-pod) | | $5.9^{a}$ | $3.4^{b}$ | $198^{a}$ | $1.7^{a}$ | - |
| | **NM** (N until maturity ) | | $5.9^{a}$ | $3.7^{a}$ | $214^{a}$ | $1.8^{a}$ | - |
| | | **CDC Greenland** | $5.1^{a}$ | $3.0^{a}$ | $163^{a}$ | $1.6^{a}$ | $62^{b}$ |
| | | **CDC Sedley** | $5.1^{a}$ | $3.0^{a}$ | $160^{a}$ | $1.3^{a}$ | $71^{ab}$ |
| | | **CDC Milestone** | $4.6^{ab}$ | $3.1^{a}$ | $149^{ab}$ | $1.7^{a}$ | $82^{a}$ |
| | | **CDC Blaze** | $4.4^{b}$ | $3.1^{a}$ | $140^{b}$ | $1.3^{a}$ | $74^{ab}$ |
| | | **CDC Rouleau** | $4.9^{ab}$ | $2.8^{b}$ | $143^{b}$ | $1.4^{a}$ | $85^{a}$ |
| M2 | **NI** (Inoculant No-N) | | $3.3^{b}$ | $2.9^{c}$ | $96^{b}$ | $1.1^{b}$ | - |
| | **NF** (N until flowering) | | $2.6^{c}$ | $2.4^{d}$ | $65^{c}$ | $1.1^{b}$ | - |
| | **NP** (N until mid-pod) | | $5.3^{a}$ | $3.9^{b}$ | $203^{a}$ | $2.2^{a}$ | - |
| | **NM** (N until maturity ) | | $4.9^{a}$ | $4.2^{a}$ | $203^{a}$ | $2.1^{a}$ | - |
| | | **CDC Greenland** | $4.3^{b}$ | $3.3^{b}$ | $150^{b}$ | $1.6^{b}$ | $48^{c}$ |
| | | **CDC Sedley** | $4.2^{b}$ | $3.1^{c}$ | $143^{b}$ | $1.6^{b}$ | $30^{d}$ |
| | | **CDC Milestone** | $3.1^{c}$ | $3.4^{ab}$ | $112^{c}$ | $1.3^{bc}$ | $39^{cd}$ |
| | | **CDC Blaze** | $3.0^{c}$ | $3.5^{a}$ | $109^{c}$ | $1.2^{c}$ | $60^{b}$ |
| | | **CDC Rouleau** | $5.6^{a}$ | $3.4^{ab}$ | $194^{a}$ | $2.4^{a}$ | $78^{a}$ |

Means followed by the same letter within the N treatments or the cultivars in each soil media are not significant at $P<0.05$

†Due to significant interactions of cultivars by N treatments, average DW, N content and yield of each cultivar under varied N treatments is presented in appendix A.

Despite the small average of lentil DW and N content in the inoculated treatments, total N content of lentil in some cultivars was comparable to reported field data. Total N content in the cultivars CDC Sedley (153 mg N plant$^{-1}$) and CDC Greenland (137 mg N plant$^{-1}$) in M1 (Table 7.4) were comparable to the average plant N content from inoculated field studies in Saskatchewan (111 mg in 2006; 96 mg in 2007; unpublished data, 2010, Zakeri and Bueckert, Department of Plant Sciences, University of Saskatchewan).

### 7.4.2 Growth prior to physiological maturity

By flowering, the average N content of NF plants was significantly greater than NI plants (Table 7.5), indicating that plant growth in the inoculated treatment was restricted by the lack of adequate starter N in both media. A wide range of 21 to 39 mg N plant$^{-1}$ in M1, and 23 to 31 mg N plant$^{-1}$ in M2 was seen in NI plants at flowering. Meanwhile, the NF plants accumulated more N than NI, and had a narrower range of 42-46 mg N plant$^{-1}$ in M1 and 34-40 mg N plant$^{-1}$ in M2 (Table 7.5). Although $N_2$ fixation was not measured prior to maturity, comparing DW and N content of NI plants prior to and after flowering suggested that maximum $N_2$ fixation in lentil occurred after flowering, and varied among different cultivars.

Table 7.5 Total plant N content (mg plant$^{-1}$) of lentil measured at first flower and mid-pod stage in response to N treatments and rhizobia inoculant. Data are averaged over five cultivars and three replicates.

| N Treatment | Cultivars | M1 | | M2 | |
|---|---|---|---|---|---|
| | | Growth stage | | Growth stage | |
| | | First - Flower | Mid-pod | First - Flower | Mid-pod |
| **NI** (Inoculant No-N) | | 32$^{b}$ | 113$^{b}$ | 27$^{b}$ | 113$^{b}$ |
| **NF** (N until flowering) | | 43$^{a}$ | 90$^{c}$ | 36$^{a}$ | 83$^{c}$ |
| **NP** (N until mid-pod) | | -- | 204$^{a}$ | -- | 195$^{a}$ |
| | **CDC Greenland** | 31$^{b}$ | 146$^{a}$ | 30$^{b}$ | 137$^{a}$ |
| | **CDC Sedley** | 42$^{a}$ | 143$^{a}$ | 36$^{a}$ | 132$^{ab}$ |
| | **CDC Milestone** | 38$^{a}$ | 146$^{a}$ | 32$^{ab}$ | 136$^{a}$ |
| | **CDC Rouleau** | 38$^{a}$ | 129$^{b}$ | 30$^{b}$ | 124$^{b}$ |
| | **CDC Blaze** | 39$^{a}$ | 137$^{ab}$ | 34$^{ab}$ | 123$^{ab}$ |

Means followed by the same letter within the N treatments or the cultivars in each soil media are not significant at $P<0.05$

As plants grew and approached the mid-pod stage (5 pod plant$^{-1}$), the ranking of N content for the N treatments changed. Sampling at mid-pod showed that the greatest N content came from NP, followed by NI and then NF. At mid-pod, plant N content was lower in NI than in NP, indicating that $N_2$ fixation could not provide as much N as the plants received in the NP treatment (Table 7.5).

Closer inspection of the cultivars revealed that maximum growth in the small-seeded cultivars was achieved earlier than in the large-seeded cultivars, with a range of responses in between. By mid-pod, cultivars showed a wider range of DW and N content due to the lower DW and N content of CDC Rouleau. In M1, CDC Greenland had a significantly lower total N content than the other cultivars at flowering, and CDC Rouleau had a significantly lower total N than three others at mid-pod. In M2, CDC Greenland and CDC Rouleau had lower N contents at flowering, and CDC Rouleau was lower than three other cultivars at mid-pod. The smallest average plant N at mid-pod across M1 and M2 was measured in CDC Greenland. Maximum growth in the large-seeded cultivars, with greater biomass, occurred later than mid-pod (Table 7.4 and 7.5). Response of cultivars to the N treatments also varied. In the third treatment, NP, CDC Milestone and CDC Blaze achieved the greatest amount with N accumulated with an average of 203 and 209 mg N plant$^{-1}$ in M1 and M2, respectively (data are not presented).

### 7.4.3 Nitrogen fixation

Averages lentil *%Ndfa* at physiological maturity was 75% in M1 and 51% in M2. The smaller *%Ndfa* of lentil in M2 than in M1 more likely associated with smaller initial N content of M2 than M1. The average initial soil N, in the form of ammonium and nitrate, was 74 and 36 μg N g$^{-1}$ soil in M1 and M2, respectively. In addition, greater soil bulk density and leaf loss (VDW lose) that were more enriched in atmospheric $N_2$ in M2 than in M1 could be associated with the varied *%Ndfa* in two runs of the experiment. When data were averaged over two soil media, CDC Rouleau had the greatest *%Ndfa*, CDC Milestone had the lowest *%Ndfa*, and the other cultivars were intermediate. Variations of the cultivars %*Ndfa* were not significant in M1, whereas a wide range of 30-78% was measured for the cultivars %*Ndfa* in M2 (Table 7.4).

**7.4.4 Plant phenology and yield formation**

Harvest index, a measure of the crop efficiency at partitioning of total DW to yield, varied among N treatments, cultivars and soil media. The greater biomass and smaller seed yield in M1 (4.8 g DW plant$^{-1}$ and 1.45 g seed plant$^{-1}$) than in M2 (4.0 g DW plant$^{-1}$ and 1.63 g seed plant$^{-1}$) resulted in lower HI in M1 compared to M2. Variability of HI among the four N treatments was only significant in M2, with the smallest HI recorded for the inoculated plants among the N treatments. Severe N deficiency in NF and occasionally in NI resulted in lower HI values in the large-seeded cultivars (CDC Greenland and CDC Sedley), whereas the average HI in small-seeded cultivars (CDC Milestone, CDC Blaze and CDC Rouleau) increased likely due to their earlier maturity and their loss of a large portion of biomass. The response of lentil HI to N treatment and soil media indicated that availability of N for plants impacted HI in two ways: 1) N deficiency increased leaf loss and as a result the proportion of seed to biomass increased, 2) greater available N increased vegetative growth more than it increased seed yield, so HI was lower as a result (Hay et al., 1995).

Seed size and number of seeds per plant varied in different N treatments. Applying N during the reproductive stage (NP and NM) increased the seed size and the number of seeds per plant compared to NF and NI (Table 7. 6). The greater average seed size in NP and NM, compared to NI and NF, came from a smaller ratio of immature seeds per plant in NP and NM. In addition, more available N in the NP and NM treatments increased the duration of reproductive growth and seed weight increased.

Table 7.6 Response of yield related parameters, phenology, and residual soil N at the end of experiment for five lentil cultivars treated with four N treatments in M1 and M2 soil media

| Media | N treatment | Cultivars | Days to Flowering | Days in reproductive growth | Days to maturity | 1000 Kernel WT | Seed Number | Residual N |
|---|---|---|---|---|---|---|---|---|
| | | | days | days | days | g | Seed plant$^{-1}$ | µg g soil$^{-1}$ |
| M1 | **NI** (Inoculant No-N) | | $33^{a}$ | $62^{b}$ | $95^{b}$ | $42^{ab}$ | $33^{bc}$ | $22^{b}$ |
| | **NF** (N until flowering) | | $33^{a}$ | $46^{c}$ | $79^{c}$ | $38^{b}$ | $30^{c}$ | $21^{b}$ |
| | **NP** (N until mid-pod) | | $32^{a}$ | $68^{ab}$ | $100^{ab}$ | $41^{ab}$ | $43^{a}$ | $22^{b}$ |
| | **NM** (N until maturity ) | | $32^{a}$ | $77^{a}$ | $109^{a}$ | $47^{a}$ | $40^{ab}$ | $65^{a}$ |
| | | **CDC Greenland** | $30^{b}$ | $68^{ab}$ | $98^{a}$ | $58^{a}$ | $27^{b}$ | $33^{ab}$ |
| | | **CDC Sedley** | $32^{bc}$ | $74^{a}$ | $106^{a}$ | $53^{a}$ | $24^{b}$ | $25^{b}$ |
| | | **CDC Milestone** | $28^{d}$ | $53^{b}$ | $81^{b}$ | $38^{b}$ | $43^{a}$ | $27^{b}$ |
| | | **CDC Blaze** | $33^{b}$ | $50^{b}$ | $83^{b}$ | $30^{c}$ | $43^{a}$ | $48^{a}$ |
| | | **CDC Rouleau** | $40^{a}$ | $61^{ab}$ | $101^{a}$ | $31^{c}$ | $45^{a}$ | $30^{b}$ |
| M2 | **NI** (Inoculant No-N) | | $33^{a}$ | $41^{b}$ | $74^{b}$ | $34^{b}$ | $34^{b}$ | $13^{c}$ |
| | **NF** (N until flowering) | | $34^{a}$ | $48^{b}$ | $82^{b}$ | $33^{b}$ | $41^{b}$ | $12^{c}$ |
| | **NP** (N until mid-pod) | | $34^{a}$ | $69^{a}$ | $103^{a}$ | $36^{a}$ | $65^{ab}$ | $20^{b}$ |
| | **NM** (N until maturity ) | | $33^{a}$ | $74^{a}$ | $107^{a}$ | $38^{a}$ | $80^{a}$ | $35^{a}$ |
| | | **CDC Greenland** | $35^{b}$ | $60^{ab}$ | $95^{b}$ | $44^{a}$ | $71^{ab}$ | $17^{ab}$ |
| | | **CDC Sedley** | $33^{bc}$ | $60^{ab}$ | $93^{bc}$ | $47^{a}$ | $35^{c}$ | $16^{b}$ |
| | | **CDC Milestone** | $27^{d}$ | $54^{bc}$ | $81^{cd}$ | $32^{ab}$ | $39^{bc}$ | $22^{ab}$ |
| | | **CDC Blaze** | $31^{c}$ | $44^{c}$ | $75^{d}$ | $27^{b}$ | $44^{b}$ | $25^{a}$ |
| | | **CDC Rouleau** | $42^{a}$ | $70^{a}$ | $112^{a}$ | $28^{b}$ | $88^{a}$ | $21^{ab}$ |

Means followed by the same letter within the N treatments or the cultivars in each soil media are not significant at $P<0.05$

The smaller number of seeds per plant in the N-deficient treatments (NF and NI) compared to the N sufficient treatments (NP and NM) was related to low availability of assimilates and N for seeds and a shorter period of seed filling. Nitrogen treatments had a greater impact on the number of seeds than on the 1000 seed weight (Table 7.6). Many seeds in the NF treatment were small and immature, and reduced the average 1000 seed weight compared to the same cultivars listed in the Saskatchewan seed guide (Saskatchewan Seed Growers Association, 2010). This difference suggested that large seeded cultivars were more susceptible to unfavorable conditions of the greenhouse (high temperature and low light intensity, root restriction, insect damage, soil nutrient supply) and method of harvest, but any conclusion based on seed weight should consider the number of immature seed.

The average number of days to flowering in lentil cultivars was not affected by N treatment or soil media (Table 7.6). But, the number of days to maturity (DTM) was significantly affected by N treatment. The earliest DTM was observed in the NF treatments, followed by the inoculated plants. Applying more N in the NP and NM treatments delayed the maturity of the lentil cultivars. Days to maturity was increased with increasing N supply, mainly via extending the time spent in reproductive growth. The time spent in reproductive growth ranked in the same order as the N supply from treatments: NF and NI, followed by NP and finally NM. Reproductive growth was about 41 to 46 d under the NF and NI treatments, and further N supply extended reproductive growth to 68 d (NP), and 75 d (NM), as in Table 7.6. Higher $N_2$ fixation of a few cultivars in either media for the NI plants provided as much N as the fully fertilized plants in NP or NM (Table 7.4), thus days to maturity in these cultivars were similar to the fully fertilized plants (Table 7.6).

CDC Milestone and CDC Blaze matured earlier than the other cultivars in both soils. CDC Rouleau, the small red-cotyledon and medium maturing cultivar, unexpectedly matured later than the other cultivars in both soils. The later maturity of CDC Rouleau in the inoculated treatment, compared to the other inoculated cultivars, increased the average of the lentil days to maturity. When delayed flowering in CDC Rouleau was taken into account, the later maturity of this cultivar did not overly extend the duration of reproductive growth in M1, but its maturity was delayed in M2. In M1, CDC Greenland and CDC Sedley had the longest duration of reproductive growth (67 d), and in M2 CDC Rouleau spent 70 d in reproductive growth.

In summary, N-sufficiency promoted full season growth, with season length varying depending on cultivar. N-deficiency at the end of the growing season induced early maturity at the expense of yield. CDC Rouleau had a similar number of days to maturity in NF, NP and NM treatments compared to the pattern seen in other cultivars, likely the long vegetative phase before flowering could accumulate sufficient N to take the plant through reproductive growth without causing premature senescence. For most of the early flowering cultivars of lentil cultivars, N application in mid-reproductive growth appears to maximize both growth and yield.

### 7.4.5 Residual soil N after harvest

The amount of unused or residual over soil N, measured after plant harvesting, varied in pots which had either different soil media or received varied N treatments (Table 7.6). The average residual soil N was 33 and 20 μg $g^{-1}$soil in M1 and M2, respectively. Average residual N in NI and NF were similarly low in all cultivars in both media. Low levels of residual N in NI and NF along with low plant N content demonstrated that plants under these two treatments were N-deficient. The average residual N from NM treatment was significantly greater than other three treatments, indicating that late-season application of N (NM) was not efficiently taken up by lentil cultivars. Differences of residual N among NP, NI and NF were not significant in M1, but residual soil N in NP was greater than NI and NF in the second soil.

Among the cultivars, CDC Blaze left more N in soil than other cultivars in both media, although differences between the CDC Blaze and other cultivars were not always significant. Variability of the residual N among cultivars was not solely associated with the days to maturity, but was related to the ability of cultivars for N uptake and biomass accumulation. Cultivar CDC Sedley (with final DW of 6.7 g plant$^{-1}$) and CDC Blaze (with final DW of 5.1 g plant$^{-1}$) had 36 and 86 μg residual N g soil$^{-1}$ in NM, respectively.

### 7.4.6 Correlations among the variables

Days to maturity also correlated with total plant N content, with the longer the growing season the greater the N uptake. Nitrogen application after flowering increased total plant N content and caused later maturity in most of the cultivars (Fig. 7.1). The association of plant N content and days to maturity in early cultivars (CDC Blaze and CDC Milestone) was not as strong as in late-maturing cultivars, likely due to their limited ability to uptake and use N later in the growing season. CDC Rouleau, another medium maturing cultivar, differed from other cultivars in days to maturity under N deficiency, likely due to its later flowering.

Lentil as an indeterminate crop improves its yield by continual flowering and pod formation during reproductive growth. Total plant N content at maturity was associated with seed yield in all N treatments and cultivars (Fig 7.1). In addition, early maturing cultivars had less response to N application after mid-pod. Inherently, a longer duration of reproductive growth in lentil should result in greater yield. Depending on cultivar and conditions, the pattern between yield and reproductive growth period was linear (r=0.92) over all data (Fig 7.1), therefore yield in some cultivars declined due to the longer reproductive growth. Plant chlorosis was the only indicator of lentil maturity in this research, and may not be a suitable method for the greenhouse conditions (such as low light intensity and a wetter soil water status) that likely prolonged plant greenness compared to field conditions.

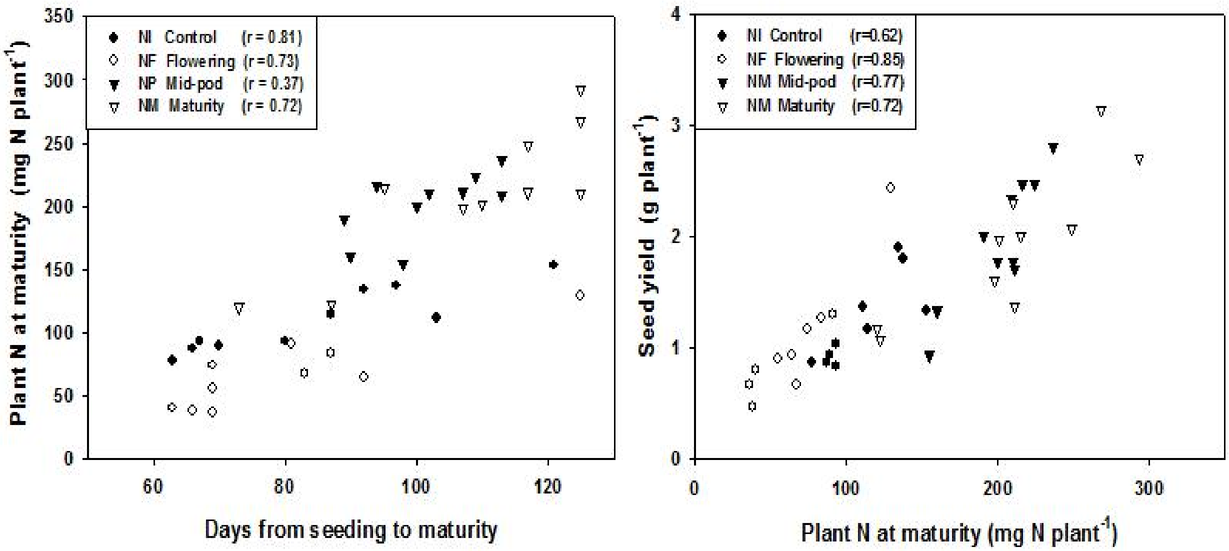


Figure 7.1 Associations of lentil plant N content at maturity with the days to maturity (upper panel), and yield and plant N content (lower panel) under four N treatments. Each data point is averaged over three replications, two soil media and five cultivars.

### 7.4.7 Biomass change during reproductive growth

Changing in lentil vegetative biomass during reproductive growth (VDW) was varied by N treatments, cultivars and soil media. In M1, late reproductive N treatments NP and NM produced >4 g plant$^{-1}$ VDW, followed by NI (2.98 g plant$^{-1}$) and then NF (2.2 g plant$^{-1}$). In M2, late reproductive N treatments NP and NM produced 3.1 and 2.8 g plant$^{-1}$ VDW, respectively, followed by NI (2.22 g plant$^{-1}$) and then NF (1.49 g plant$^{-1}$). The NI and NF plants either produced only half the weight of leaves and stems than NP and NM in late reproductive growth due to an increased demand for remobilized assimilate, or NI and NF plants lost substantial weights of leaf due to premature senescence and leaf loss. Cultivars also differed in VDW during the seed filling. In M1, cultivars lost a range of 2.98 to 3.80 of VDW, and in M2 they lost 1.75 to 3.25 g plant$^{-1}$. Maximum DW and N content of CDC Milestone and CDC Blaze, were achieved earlier than the later maturing cultivars, and late harvesting of these cultivars contributed to leaf loss.

### 7.5 Discussion

Similar to results from earlier studies on lentil and other legumes, N uptake in lentil lasts until at least the mid-pod stage, but the exact time or growth stage when $N_2$ fixation ends is usually not recorded in published studies. In lentil, $N_2$ fixation and N uptake after flowering differed among cultivars (Kurdali et al., 1997), but depending on environment N accumulation could continue until early pod formation (van Kessel 1994). Although N applied after mid-pod up to maturity did not improve yield, one cultivar, CDC Greenland, was able to produce more yield under late N application (NM). The greater DW and N accumulation from NM in this later maturing lentil is in agreement with soybean. Later maturing cultivars of soybean have more DW and uptake more N after flowering and produce more yield than early maturing cultivars (Isfan 1991).

From other field studies, lentil obtains most of its N requirements through $N_2$ fixation (Kurdali et al., 1997; van Kessel 1994). Significant cultivar differences in yield in NP and NM compared to NI suggested that $N_2$ fixation provided less N overall than the fertilized lentil. The greenhouse conditions could have limited fixation via a wet soil, or yield can be increased if *%Ndfa* is improved. When legume crops rely on $N_2$ fixation, lentil had a lower N concentration than chickpea, cowpea (*Vigna unguiculata*) and pea (*Pisum sativum*) crops (Ayaz et al., 2004). Lentil has a smaller biomass than the other pulses, and a lower N concentration, both of which suggest that growth and yield in lentil is limited by a limited N supply from fixation. By comparing the distribution of N and DW in lentil in varied environments, Whitehead et al. (2000) concluded that lentil yield, especially in older cultivars, was restricted by inadequate N.

Late-season N application, after flowering, has been suggested to provide more N to legume crops while having less inhibitory effects on $N_2$ fixation. Substantial competition of seeds for N in addition to accumulated plant carbohydrate after plant flowering could reduce the negative effects of nitrate on nitrogenase activity (Salvagiotti et al., 2008). Applying N after flowering to inoculated soybean in the field (Gan et al., 2003) and in the greenhouse (Isfan 1991) increased yield, likely due to a longer photosynthesis and growth period as well as an extended period of $N_2$ fixation. Conversely, lack of response or a similar yield response to N application after flowering in inoculated soybean is due to the crop already having adequate $N_2$ fixation (Gutiérrez-Boem et al., 2004), and so this strategy may not be profitable in all years. Applying N after flowering in the current study improved lentil yield in late maturing cultivars more than in early maturing cultivars, similar to soybean (Isfan 1991). A further strategy would be to seek strains that would produce earlier nodulation and N fixation for the host crop. In legume crops, seeds are the major sink that compete with nodules for assimilates and nutrients. In pea, the priority of assimilate distribution to nodules was decreased and became equal to that of the shoot by seed-filling, indicating that availability of assimilates to nodules would also decline in mid to late reproductive growth (Voisin et al., 2002).

Some cultivars had similar %*Ndfa* and total N fixed to other field studies of lentil. Van Kessel (1994) measured 6500 kg DW $ha^{-1}$ and 149 kg N $ha^{-1}$ at 181 days after planting (this is equal to 6.5 g DW and 149 mg N $plant^{-1}$, assuming 100 plants $m^{-2}$) from one cultivar of lentil under irrigated conditions in Saskatchewan. From Syria and under rainfed conditions, five cultivars of lentil averaged 193 kg N $ha^{-1}$ at late podding, with 123 kg N $ha^{-1}$ of this from $N_2$ fixation (Kurdali et al., 1997). However, overall $N_2$ fixation was restricted by conditions in the greenhouse such as limited root extension, warmer air and soil temperature. Limited initial soil N, especially in M2, was also associated with reduced %*Ndfa*. The later flowering of CDC Rouleau provided a longer time frame for root extension and nodule formation prior to flowering; hence, it had greater %*Ndfa* than other cultivars.

As an indeterminate crop, lentil continued producing biomass, flowers and pods after first flowering in response to more available N in NP and NM. Days to flowering was not affected by N treatments and soil media, but varied among the cultivars. Time to flowering in lentil is a linear function of photoperiod and temperature (Summerfield et al., 1985; Erskine et al., 1994). Unlike days to flowering, the total crop life cycle (days from seeding to maturity) varied among the N treatments, cultivars and soil media. The extended growth duration and higher yield due to N applied up to mid pod or maturity (NP and NM) agreed with other studies where a longer life cycle correlated with higher yield (Ayaz et al., 2004, Summerfield et al., 1985, Whitehead et al., 2000).

Greater N uptake before flowering in CDC Rouleau (NF and NI) than other cultivars indicated that N supply up to flowering in a late-flowering cultivar could maintain lifecycle length. Nitrogen supply up to flowering would usually result in a premature reproductive phase in earlier flowering cultivars. CDC Rouleau had similar days to maturity in both N-deficient (NF and NI) and N-sufficient (NP and NM) treatments, demonstrating that substantial pre-flowering N supply could be adequate in some cultivars. The lentil cultivars formerly grown widely in Saskatchewan, Laird and Eston had a medium flowering date (53 d) and very late maturity (Laird), or early flowering (48 d) and early maturity (Eston). Most cultivars released in the late 1990s and 2000s have early flowering (<50 days) and earlier maturity dates, although CDC

Greenland has a later maturity date (medium late) along with most large-green market class cultivars, when compared to many other cultivars from the most recent decade. In addition, early maturing cultivars had less response to N application after mid-pod. Inherently, a longer duration of reproductive growth in lentil should result in greater yield.

### 7.6 Conclusion

Application of N during the crop life cycle improved plant DW, N content and seed yield in lentil. Nitrogen supply only up to flowering was inadequate to produce yield, and lentil therefore required substantial N supply from uptake or $N_2$ fixation during reproductive growth to generate growth and yield. Application of N up to the mid-pod stage (NP) demonstrated that this treatment supplied adequate N for growth and yield. The NP treatment was more efficiently used by the plant for yield than the later N application in NM.

The longer DTM of lentil in response to the post-flowering N fertilizer occurred due to an extended period of reproductive growth, not to any changes in the longer days to flowering. Nitrogen applied up to maturity tended to extend the days to maturity, to reduce HI and even reduce yield. Late flowering, as seen in the cultivar CDC Rouleau, enabled this type of cultivar to provide more N in the vegetative phase prior to flowering, sustain a normal life cycle length, and produce adequate yield. We found current cultivars of lentil for Saskatchewan and western Canada require N supply up to at least the mid-pod stage, although the exact stage and timing warrants more detailed study, specifically in the field.

# 8.0 Modeling Lentil Leaf N, Using the SPAD Chlorophyll Meter

## 8. 1 Abstract

Leaf chlorophyll and leaf N have been frequently used to model plant total N and yield. However, in $N_2$-fixing crops with indeterminate growth, the association of leaf N with total plant N and yield can be unpredictable. Prolonged N fixation can lower variation of leaf N among different traits, whereas post-flowering leaf production may alter the leaf N-yield association. Leaf chlorophyll content of the uppermost leaves of lentil (*Lens culinaris* Medic.) was estimated by a SPAD chlorophyll meter in the field. Then, leaf area, leaf thickness and leaf N concentration were measured in the laboratory. Leaf N concentration was compared with the entire plant N status and yield, and SPAD values were used to model leaf N concentration. Data came from different environments and N availability conditions in Saskatchewan.

Leaf N concentration had responded similarly to soil N availability as total plant N and yield. At physiological maturity, correlation of leaf N concentration was strong with final plant DW (0.70), stem N concentration (0.58) and yield (0.52), but not with concentration of N in total leaf biomass. Correlation of SPAD-leaf N was increased from 66% to 76%, when SPAD values were adjusted for leaf thickness. A linear regression model predicted 56%, and a non-linear neural network model predicted 83% of the variation of leaf N concentration. Results demonstrated that leaf N can represent soil and plant N status, and SPAD chlorophyll meter can be used to predict leaf N concentration in lentil, especially after pod-set.

## 8. 2 Introduction

Nitrogen is known as the most limiting factor for plant growth in natural ecosystems (Aerts, 1990). In croplands, where N management can maximize yield, various standards have been developed to determine the response of plant to N. Among various plant characteristics, strong associations of leaf photosynthesis and leaf N content (Charles-Edwards et al., 1987) frequently facilitate modeling of plant growth and yield formation (Hikosaka, 2005; Gastal and Lemaire, 2002; Borrell et al., 2001; Evans, 1989). In soybean (*Glycin max* L.), leaf photosynthesis was closely related to atmospheric $N_2$ fixation (Vollmann et al., 2011). In sorghum (*Sorghum bicolor* L.), plant maturity was modeled by leaf photosynthesis, leaf senescence and leaf N content (Borrell et al., 2001). In soybean, late maturing cultivars prolonged photosynthesis and improved leaf N compared to early maturing cultivars (Gordon et al., 1982).

Despite the strong association of leaf N to leaf photosynthesis, this association may require adjustments for leaf area (Hikosaka, 2005), leaf thickness (Borrell et al., 2001; Chapman and Barreto, 1997), plant growth stage (Vollmann et al., 2011), and other environmental conditions (Magliette et al., 1995). Grindlay, (1997) showed that variation in specific leaf weight (SLW), *i.e.* weight per unit leaf area, under N deficiency can alter the association of leaf N and photosynthesis. Reduction in leaf thickness, SLW, and leaf N with canopy depth is attributed to lack of sufficient light reaching the base of canopy (Gastal and Lemaire, 2002); SLW and leaf area (Campbell et al., 1990); and leaf N deficiency (Hikosaka et al., 1994; Borrell et al., 2001). Young leaves at the plant apex, especially in dicots that continuous canopy expansion reduces light penetration to the lower layers, may provide less variable and comparable observations (Vollmann et al., 2011; Lemaire et al., 1991). Leaf dry weight (DW), as an effective variable on leaf N concentration, can be avoided by converting leaf N concentration to specific leaf N (SLN), *i.e.* amount of N per unit leaf area (Hikosaka, 2005; Wullschleger and Oosterhuis, 1990; Koch et al., 1988)). Again, variability of SLN under varied light intensities is smaller compared to the most recent expanded leaves at the top of canopy (Vollmann et al., 2011; Campbell et al., 1990).

Reliable models on leaf N-plant growth and yield lead researchers to seek inexpensive rapid measurements of leaf N. The Soil Plant Analysis Development (SPAD) chlorophyll meter, which was first used to estimate leaf chlorophyll in rice (*Oryz sativa* L.) in Japan, has been extensively applied to leaf N estimation in different crops around the world. Esfahani et al. (2008) predicted leaf N concentration of rice by SPAD data. Similar investigations provided reliable estimates for leaf N in sorghum and pigeon pea (*Cajanus cajan* L.), corn and soybean (Zhang et al. 2009; Shafagh-Kolvanagh et al., 2008; Yamamoto et al., 2002). When associations between SPAD readings and leaf N concentration have been unsatisfactory, including measurements of leaf thickness and leaf position in models improved SPAD correlations with leaf N (Borrell et al., 2001). Although most SPAD studies have aimed at leaf N estimation, further correlations between SPAD and plant biomass, seed yield and even $N_2$ fixation is likely (Vollmann et al., 2011; Esfahani et al., 2008; Borrell et al., 2001)

Lentil is an $N_2$-fixing crop and is usually grown without additional N fertilizer. However, researchers have occasionally tried to improve lentil yield via soil N management (Bremer et al., 1989; Cowell et al., 1989; Gan et al., 2005). None of these studies estimated leaf N status as an indicator of plant response to the available N. In the current study, responses of leaf chlorophyll content, leaf N concentration and leaf thickness to varied environmental conditions and soil N status during lentil growth was investigated. Then, associations of the leaf characteristics with lentil growth, N status and yield were tested. Finally, the possibility of using a SPAD chlorophyll meter as a rapid non-destructive method of leaf N assessment was tested.

In addition to linear correlation and regression analysis, SPAD data were used to fit a non-linear model for leaf N estimation, using a neural networks (NN) model, which is capable of providing more accurate predications than simple linear regression (Yi et al., 2007). Unlike linear regression models, which produce prediction outputs by computing the intra-variability of parameters, a NN model computes both within- and between-associations of data (inputs) and produce all forms of outputs, including prediction outcomes. By computing inter-association of inputs, an NN model develops neurons which can justify the association of inputs and outputs (Pereira et al., 2009).

## 8. 3 Materials and Methods

### 8.3.1 Environment and N treatments

Leaf nitrogen concentration, leaf thickness, leaf area and leaf chlorophyll content of lentil were measured in lentil trials in four environments of Saskatchewan in 2006 and 2007. These environments were located at Saskatoon, SK (52º N and 106º W) and Indian Head, SK (50° N and 103º W). Exact locations of the experiments were Goodale research farm in 2006 (GD) and Skarsgard field in 2007 (SKA), both located within 30 km radius of Saskatoon; and Vale Farms around 40 km east of Indian Head , SK in both 2006 (IH06) and 2007 (IH07). Rainfall distribution and monthly average temperature varied among the environments. Similarly, soil N content and cultivation history in the experimental fields affected lentil growth and yield (see Table 4.1 and 4.2 in Chapter 4, and Fig. 6.1 in Chapter 6).

In Saskatoon (GD and SKA) eight cultivars of lentil were grown under three fertility treatments of 50 kg N ha$^{-1}$, granular rhizobium inoculants and a non-treated control. In Indian Head, five other cultivars of lentil were subjected to two no-tillage (NT) durations of 25-year NT (long-term: LN) and 5-year NT (short-term: SN). Finally, one late maturing cultivar of lentil, CDC Sedley, was grown under four rates of 0, 15, 30 and 60 kg N fertilizer ha$^{-1}$ at both the SN and the LN locations in Indian Head. More details regarding these trials are found in Chapters 4, 5 and 6.

### 8.3.1 Data collection

Leaf measurements were carried at three stages of vegetative growth (Veg: up to node 12), first-pod (FP: lentil had at least one pod in plot) and late-pod (LP: when the canopy started turning yellow). Measurement time was limited to between 10:00 to 12:00 h each day, when possible, to avoid variation associated with leaf starch concentration. Leaf chlorophyll content was estimated by a SPAD chlorophyll meter (Model 502 Konica Minolta Sensing, Inc, Japan) from the two to three uppermost leaves of four to six lentil plants per plot. The leaves were detached from the plant immediately and transferred on ice to a refrigerator for further measurements the next day.

In the laboratory, leaf surface area was measured using a leaf area meter (Model LI-3100C Portable Leaf Area Meter, LiCor, Lincoln, NE). Leaves then were dried at 60ºC for 24 hrs, weighed and ground for N concentration measurement by combustion using a Leco carbon-nitrogen determinator (LECO CNS 2000, St. Joseph, MI, USA). Specific leaf weight (SLW) was calculated as the ratio of dry weight to leaf area and specific leaf nitrogen (SLN) was calculated as the ratio of leaf N content (leaf DW × leaf N concentration) to leaf area. Adjusted SPAD for leaf thickness ($SPAD_{adj}$) was calculated as the ratio of the SPAD value to SLW.

### 8.3.2 Data analysis

Collected data were first analyzed for the effect of N treatments and cultivars for each growth stage and environment. In Saskatoon, data were analyzed for each environment (GD and SKA) separately to avoid confounding effects of rainfall distribution and soil conditions on results. Data were analyzed as a nested design in Proc Mixed of SAS, version 9.2 (SAS Institute, Cary, NC) with the main plots as N fertility treatments (inoculant, N fertilizer, control), the sub-plots as cultivars. Year and replication were random terms in the ANOVA model. In Indian Head, data were analyzed for the effect of NT duration (SN and LN) and cultivar (or N rates) for each growth stage. Again, data were analyzed as nested design with NT as main effect and cultivars (or N fertilizer rates) as sub-plots. In this location, year was as a fixed variable and replication was random.

Subsequently, data were pooled for all environments, treatments, growth stages and cultivars. The three growth stages of vegetative growth, first-pod and late-pod were arbitrary considered as 1, 2 and 3, respectively. Yield , harvest index (HI), days to maturity, and N concentration in total plant leaf, stem and pod were combined with the leaf properties for correlation and regression analyses. Correlation analysis was conducted for leaf parameters and plant growth and yield, using Proc CORR in SAS. Linear regression was estimated by Proc GLM in SAS for predicting leaf N concentration or SLN as dependent variables and different combinations of SPAD readings (or $SPAD_{adj}$), SLW and growth stages as independent variables. Finally, a non-linear regression was developed by a feed forward neural network model in Matlab. The non-linear model was used to predict leaf N concentration or SLN based on different combinations of SPAD (or $SPAD_{adj}$), SLW and growth stages as independent variables.

## 8. 4 Results

### 8.4.1 Pattern of leaf N

Leaf N concentration and SLN both declined during vegetative growth to late-pod, but SLW continued to increase during this period (Fig 8.1), suggesting that the late-season formed leaves were depleted in N. The greatest variations in the leaf parameters at FP and LP (when lentil had its maximum biomass and N accumulation rates) showed that leaf measurements could be limited to the after flowering growth stages (Fig. 8.1).

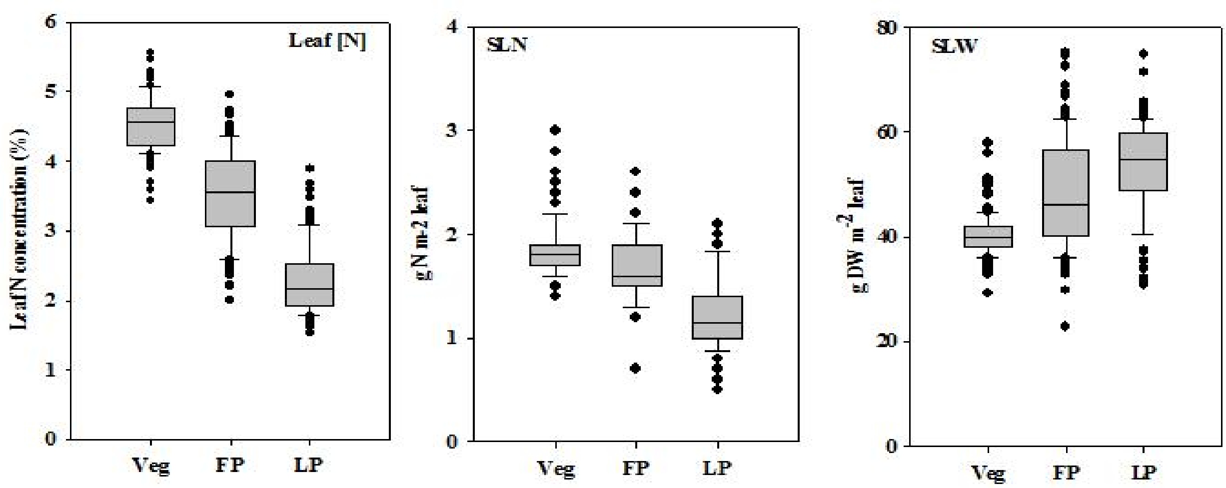


Figure 8.1 Ranges of Leaf N concentration, SLN and SLW of lentil at three stages of vegetative growth (veg), first-pod (FP) and late-pod (LP) across environments, N treatments, cultivars and growth stages. Box length represents 75% of data, the center line is median, error bars show the smallest and the largest observations, and dots are outlier data.

## 8.4.2 Environment effects

Leaf N concentration and SLN were maximized at GD, followed by SKA and then were similar at IH06 and IH07 (Fig 8.2). At GD and SKA, allying 50 kg N $ha^{-1}$to one third of the lentil plots increased the average of leaf N concentration and SLN. Smaller leaf N concentration and SLN of lentil at SKA than GD was caused by drought stress at SKA. Unlike leaf N and SLN, averages SLW at SKA, IH06 and IH07 was greater than at GD. Thicker leaves (greater SLW) could be a response to limitted leaf growth due to stress or limitted leaf expansion due to less availbale N.

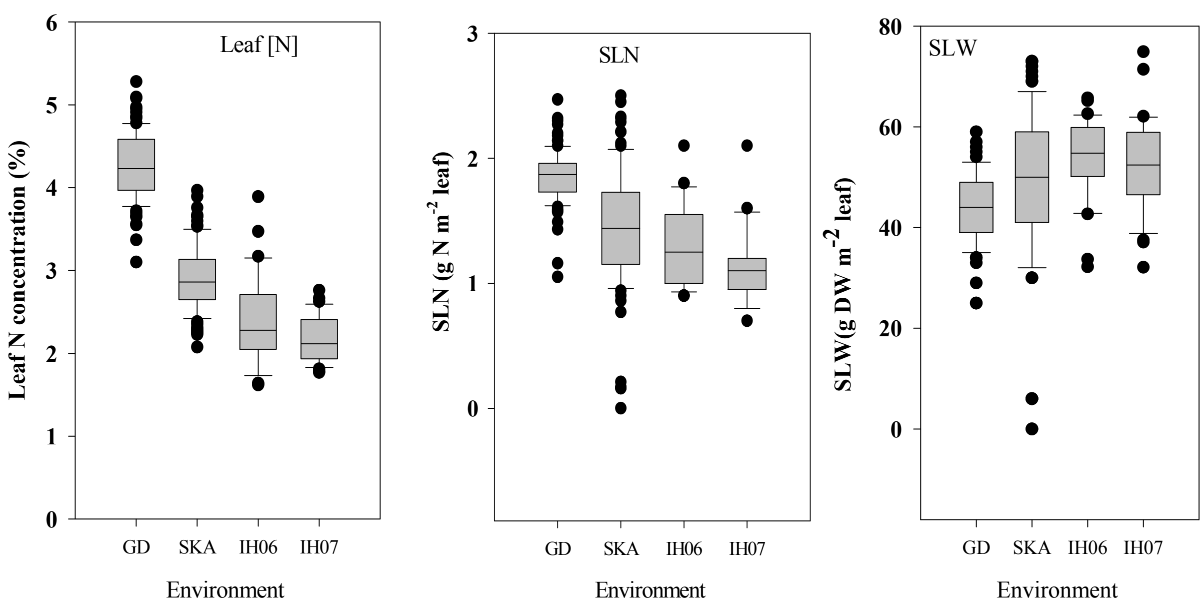


Figure 8.2 Ranges of leaf N concentration, SLN and SLW of lentil in four different environments. Data associated with four rates of N in Indian Head were omitted from this figure. Box lengths represent 75% of data and the centerline is the median and error bars show the smallest and the largest observations and dots are outlier data.

Averages of each of the three leaf measurements in the N fertilizer experiment at Indian Head, where lentil was grown with four N rates, were similar to IH06 and IH07. This similarity, which was in agreement with lentil biomass and yield at this location, suggesting that soil N and lentil $N_2$ fixation at was adequate to support lentil growth without additional N requirements. As in Fig.8.2, leaf properties spreaded in a wider range at GD and SKA than at Indian Head, possibly becouse of a broader range of cultivars and N availabilities.

### 8.4.3 Fertility treatments

The three N fertility treatments of inoculant, 50 kg N fertilizer $ha^{-1}$ and control at GD and SKA affected leaf N and leaf DW; however, the effects varied by environments and growth stages. At GD, applying N fertilizer treatment maximized leaf N concentration and SLN at first-pod only, whereas SLW remained identical across the fertility treatments throughout the entire growing season (Table 8.1). Competition of pods and leaves for N may diminish the variation of leaf N among the fertility treatments, which observed at first-pod.

Table 8.1 Average leaf N concentration, SLN and SLW of eight cultivars of lentil at three stages of vegetative growth (Veg), first-pod (FL) and late-pod (LP) under three fertility treatments at GD and SKA.

| | N Fertility Treatments | Leaf N (%) | | | SLN (g N $m^{-2}$ leaf) | | | SLW (g DW $m^{-2}$ leaf) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Veg | FP | LP | Veg | FP | LP | Veg | FP | LP |
| GD 2006 | Control | 4.5† | $4.0^{b}$ | 4.5 | 1.6 | $1.6^{b}$ | 1.9 | 34 | 40 | 42 |
| | N Fertilizer | | $4.3^{a}$ | | | $1.8^{a}$ | | | | |
| | Inoculant | | $4.1^{b}$ | | | $1.7^{b}$ | | | | |
| SKA 2007 | Control | $3.6^{c}$ | $4.7^{a}$ | $3.1^{a}$ | $1.6^{b}$ | $2.7^{a}$ | $1.7^{a}$ | $45^{a}$ | 57 | $56^{a}$ |
| | N Fertilizer | $4.0^{b}$ | $4.0^{b}$ | $2.8^{b}$ | $1.6^{b}$ | $2.4^{b}$ | $1.4^{b}$ | $41^{b}$ | | $52^{ab}$ |
| | Inoculant | $4.2^{a}$ | $4.5^{a}$ | $2.9^{ab}$ | $1.8^{a}$ | $2.6^{a}$ | $1.4^{b}$ | $43^{a}$ | | $49^{b}$ |

Means followed by same letter within columns indicate non-significant difference among the N fertility treatments in each environment ($P<0.05$)
†Non-significant effects of the fertility treatments were averaged and not indicated by a letter.

At SKA, leaf N concentration and SLN differed by both the fertility treatments, and leaf thickness (SLW). At vegetative growth stage, inoculated lentil had the greatest leaf N concentration, followed by the fertilized lentil and then the non-treated control, indicating the effect of soil N availability on leaf N concentration. At this stage (Veg), N deficient treatments (control and inoculant) had thicker leaves than the N sufficient treatment (N fertilizer). As plants grew, the pattern of leaf N among the treatments was almost reversed and leaf N concentration was maximized in the control, whereas SLW remained constant among the treatments. In the last measurement at late-pod, lentil leaves in the treated plots were more depleted in N (smaller SLN) than in the control. Averages of leaf N concentration, SLN and SLW in response to the fertility treatments totally differed from the response of total lentil DW and N and seed yield at SKA (Table 4.3, Chapter 4). Varied patterns of leaf N concentration, SLN and SLW between GD and SKA suggested that leaf N could show plant N status under varied soil and weather conditions.

### 8.4.4 N fertilizer rates

Lentil cultivar CDC Sedley, was grown under four N fertilizer rates at both SN and LN fields in Indian Head. In this experiment, average leaf N concentration and SLN both declined from FP to LP by 49 and 26%, respectively, but SLW was increased by 40% during the same period (data not presented). Leaf N concentration and SLN failed to respond to the N fertilizer rates at any growth stage, including late-pod (Fig 8.3). Slightly lower leaf N concentration and SLN with 30 kg N $ha^{-1}$ compared to other treatments could be associated with expanded leaf area in the 30 kg N $ha^{-1}$ treatment. A wider range of leaf N concentration compared to the narrow range of SLN (Fig 8.3) suggested that CDC Sedley expanded leaf area with 30 kg N $ha^{-1}$. Similar to the leaf response, total plant DW and N content as well as seed yield of lentil did not respond to the N fertilizer rates (Chapter 6).

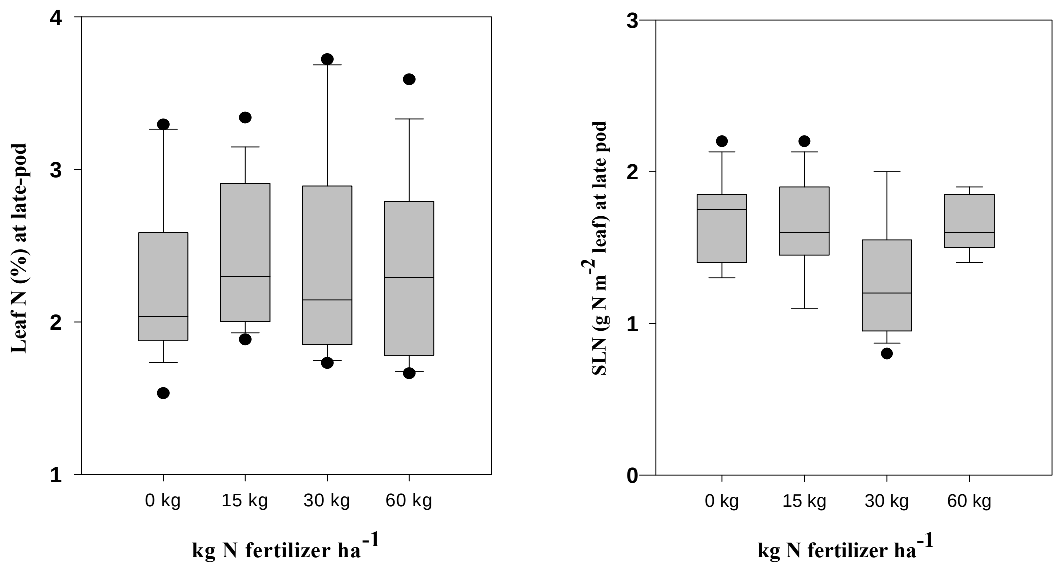


Figure 8.3 Range of leaf N concentration, SLN and SLW of lentil under four different N fertilizer rates in Indian Head. Data are from two tillage systems and three replications. Box length represents 75% of data, the centerline is the median, error bars present the smallest and the largest observations, and dots are outlier data.

### 8.4.5 No tillage duration

Soil available N (nitrate and ammonium) at seeding was greater in LN than in SN (Table 6.1, Chapter 6). The varied N availability due to the NT duration appeared in the leaf N concentration measurements; however, the effects varied by year and growth stage (Table 8.2). Greater leaf N concentration and SLN of lentil in SN than in LN at vegetative growth and late-pod in 2006 were in agreement with response of yield and total plant N to the tillage duration. In fact, total biomass differed by the NT duration at podding only in 2006, but leaf N seemed to be more sensitive to the N availability than total plant N. In 2007 (IH07), leaf N concentration in LN was greater than in SN at first-pod only. The varied leaf N concentration appeared to be associated with leaf area, because SLN did not differ due to NT duration in this year. In this experiment, where lentil was grown without N fertilizer, leaf thickness was identical in both SN and LN (Table 8.2)

Table 8.2 Average leaf N concentration (Leaf N), SLN and SLW of lentil in three stages of vegetative growth (Veg), first-pod (FL) and late-pod (LP) in two tillage systems of LN and SN during 2006 and 2007. Data associated with the four N fertilizer rates in this location were omitted from analysis of variance.

| | | **Leaf N** (%) | | | **SLN** (g N m$^{-2}$ leaf) | | | **SLW** (g DW m$^{-2}$ leaf) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Year** | | **Veg** | **FP** | **LP** | **Veg** | **FP** | **LP** | **Veg** | **FP** | **LP** |
| **2006** | **LN** | $4.6^{b}$ | $3.6^{ab}$ | $1.8^{b}$ | $1.9^{b}$ | $1.8^{b}$ | $1.1^{ab}$ | $41^{b}$ | $46^{b}$ | $56^{a}$ |
| | **SN** | $5.0^{a}$ | $4.0^{a}$ | $2.3^{a}$ | $2.3^{a}$ | | $1.3^{a}$ | $45^{a}$ | | $51^{ab}$ |
| **2007** | **LN** | $4.5^{b}$† | $3.3^{b}$ | $1.8^{b}$ | $1.7^{c}$ | $2.0^{a}$ | $1.1^{a}$ | $38^{b}$ | $67^{a}$ | $49^{b}$ |
| | **SN** | | $2.9^{c}$ | | | | | | | $54^{ab}$ |

Means followed by same letter within columns indicate non-significant difference among the N fertility treatments in each environment ($P<0.05$)
†: Non-significant differences by NT duration were averaged for each year.

### 8.4.6 Lentil cultivars

In Saskatoon, lentil cultivars differed in their leaf N concentration, SLN and SLW at late-pod, with a greater variation at GD than SKA (Table 8.3). At GD, CDC Red Rider had the greatest leaf N concentration, one of the smallest SLN and the lowest SLW among the eight lentil cultivars at late-pod. This cultivar, likely, maintained high leaf N concentration by reducing leaf thickness. At late-pod, CDC Blaze had the smallest leaf N concentration, but the greatest SLW among the cultivars at GD (Table 8.3). At SKA, where lentil growth was severely restricted by a mid-season drought stress, variation in the cultivars leaf properties diminished at late-pod, except for CDC Blaze that had a lower leaf N concentration than the other cultivars (Table 8.3). CDC Blaze matured before most cultivars and was most likely in a more advanced growth stage than the other cultivars at the time of sampling.

At IH, where five other cultivars of lentil were grown in two tillage systems of SN and LN, cultivars leaf N concentration and SLN did not differ at any growth stages. Among the five cultivars, CDC Robin and CDC Vantage had the lowest (49 g $m^{-2}$ leaf) and CDC Sedley had the greatest (58 g $m^{-2}$ leaf) SLW. Two cultivars, CDC Milestone and CDC Redcap, did not have different SLW values. CDC Robin produced one of the greatest yields and had a larger plant N content among the cultivars.

Table 8.3 Averages leaf N concentration, SLN and SLW of eight cultivars of lentil grown at GD and SKA under three fertility treatments at late-pod.

| **Cultivar** | **Leaf N** (%) | | **SLN** (g N $m^{-2}$ leaf) | | **SLW** (g DW $m^{-2}$ leaf) | |
|---|---|---|---|---|---|---|
| | **GD06** | **SKA07** | **GD06** | **SKA07** | **GD06** | **SKA07** |
| CDC Blaze | $4.2^{d}$ | $2.7^{b}$ | $2.0^{a}$ | $1.6^{ab}$ | $49^{a}$ | $57^{ab}$ |
| CDC Greenland | $4.5^{bc}$ | $3.0^{ab}$ | $1.7^{c}$ | $1.5^{ab}$ | $38^{de}$ | $48^{bc}$ |
| CDC Milestone | $4.5^{bcd}$ | $2.8^{ab}$ | $2.0^{a}$ | $1.5^{ab}$ | $45^{ab}$ | $53^{abc}$ |
| CDC Plato | $4.5^{bc}$ | $3.0^{ab}$ | $1.8^{bc}$ | $1.3^{b}$ | $40^{cde}$ | $43^{c}$ |
| CDC Red Rider | $4.9^{a}$ | $3.1^{a}$ | $1.7^{c}$ | $1.5^{ab}$ | $35^{e}$ | $49^{abc}$ |
| CDC Rouleau | $4.4^{bcd}$ | $2.9^{ab}$ | $1.9^{abc}$ | $1.6^{a}$ | $43^{bc}$ | $57^{ab}$ |
| CDC Sedley | $4.3^{cd}$ | $2.8^{ab}$ | $1.9^{ab}$ | $1.6^{ab}$ | $45^{ab}$ | $58^{a}$ |
| CDC Viceroy | $4.6^{ab}$ | $3.1^{ab}$ | $1.9^{ab}$ | $1.6^{ab}$ | $42^{bcd}$ | $51^{abc}$ |

Means followed by same letter within columns indicate non-significant difference among the N fertility treatments in each environment ($P<0.05$)

### 8.4.7 Leaf properties and lentil yield

Leaf N concentration, measured at the canopy apex, could represent 60% of the variation in N concentration in the entire leaf biomass, measured in a sub-sample taken from the entire plant leaves, at vegetative growth (Table 8.4). This correlation was not strong after flowering (Table 8.4). Again, a negative correlation (r= - 0.37) of leaf N concentration with total plant N at late-pod showed that N accumulation in the most recently grown leaves did not follow the same pattern as the whole plant (Table 8.4). The varied association of leaf N concentration and total plant leaf N concentration suggested that leaf N concentration was affected by leaf position. The positive association of leaf N to yield toward maturity suggested that late-season leaf N measurements could be used to predict yield. At maturity, $N_2$ fixation and N uptake of lentil was expected to be less. Hence, leaf N concentration may better represent the whole plant N status. Lentil DW and yield had stronger correlation with leaf N concentration at GD than SKA (data not presented).

Table 8.4 Correlation coefficients of leaf N concentration, SLN and SLW with different variables at vegetative growth (Veg), first-pod (FP) and late-pod (LP).

| | Leaf N (%) | | | SLN (g N $m^{-2}$ leaf) | | | SLW (g DW $m^{-2}$ leaf) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Veg | FP | LP | Veg | FP | LP | Veg | FP | LP |
| SLN | - | 0.45 | 0.54 | | | | | | |
| SLW | -0.53 | - | -0.48 | 0.69 | 0.87 | 0.49 | | | |
| Yield | 0.48 | - | 0.52 | - | -0.50 | - | -0.51 | -0.48 | -0.37 |
| Harvest index | - | - | - | - | - | - | - | - | - |
| Total leaf N † | 0.64 | - | - | 0.46 | 0.32 | - | -0.69 | -0.39 | - |
| Total Stem N †† | - | - | 0.58 | -0.44 | -0.31 | - | -0.72 | -0.40 | -0.35 |
| Total Pod N ††† | - | - | 0.48 | - | -0.56 | - | - | -0.56 | - |
| Total plant DW | - | | 0.69 | 0.30 | - | - | - | - | -0.39 |
| Total plant N | 0.51 | - | -0.37 | -0.39 | -0.40 | - | -0.73 | -0.48 | - |

- Only coefficients larger than ±0.30 are presented
† , †† and ††† are concentration of N in total plant leaf, stem and pod, respectively, recorded in 5 plants per plot within 7-10 days from sampling for the leaf variables.

The correlation of SLN with lentil yield was significant at vegetative and first-pod stage (Table 8.4). At these stages, SLN was negatively associated with yield (r=-0.50) and pod N concentration (r=-0.56). The negative association of SLN and yield may indicate that late-season leaf expansion was not in favor of yield. Thicker leaves were found in plants that had low N concentration in stem (r=-0.72), total leaf (r=-0.69) and total plant biomass (r= - 0.73).

### 8.4.8 SPAD chlorophyll meter

Leaf chlorophyll content, measured by SPAD chlorophyll meter, varied by environment, growth stage and N availability, but was barely affected by cultivars. Average SPAD values were almost identical amongst the environments at vegetative growth (~ 30). At first-pod and late-pod, SPAD values were maximized in trials that received N fertilizer. By these stages, trials in Saskatoon (GD and SKA) and one at Indian Head with four N rates had greater SPAD values (~ 36) than lentil at Indian Head without N fertilizer (~25). Later, averages SPAD values again became similar across the environments (~ 23) at late-pod, except for GD that SPAD values remained greater than the other locations until late pod.

In response to the fertility treatments at GD, lentil in the control treatment had smaller SPAD values than in the other two treatments. AT SKA, effects of the fertility treatments on SPAD values among the treatments existed throughout the entire growth of lentil. Although SPAD values were greater in treated plots than in the control at the first two growth stages, this pattern was reversed later, and lentil in control treatment had greater SPAD values than in treated plots at late-pod (Fig 8.4). At IH, SPAD readings at both first-pod and late-pod in SN was 6 unites greater than in LN in 2006. In the succeeding year, 2007, SPAD readings did not differ by the no-till duration. Again, four rates of 0 to 60 kg N $ha^{-1}$ did not change SPAD values in cultivar CDC Sedley in the same location (data not presented).

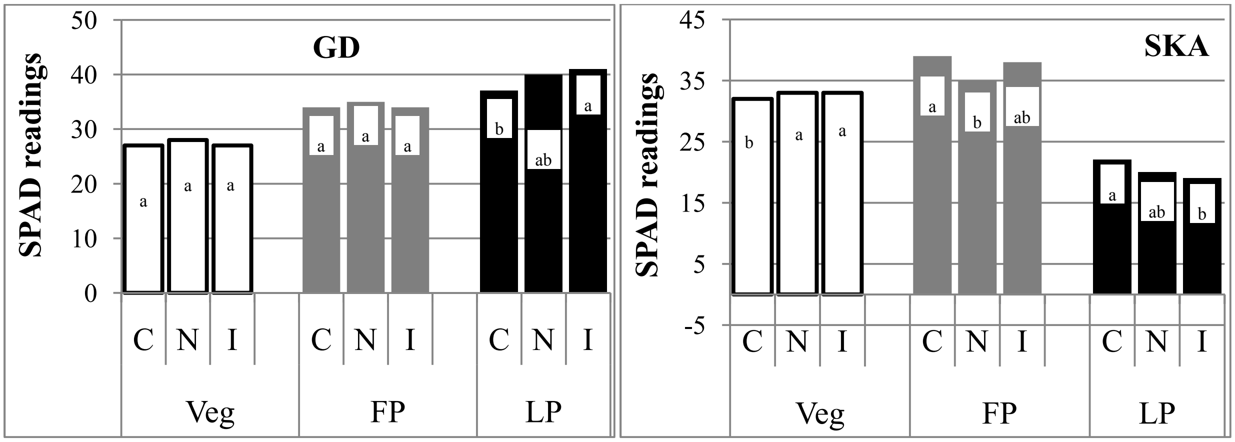


Figure 8.4 Average SPAD readings in lentil grown under three fertility treatments of Control (C), N fertilizer (N) and inoculant (I) at three stages of vegetative growth (Veg), first-pod (FP) and late-pod (LP) at GD (left) and SKA (right). Similar letters among bars charts indicates non-significant differences of SPAD amongst the fertility treatment at each growth stage-environment ($P<0.05$).

### 8.4.9 Correlation of SPAD- leaf N

SPAD values could explain 50% of the variation of leaf N concentration, when data from environments, growth stages and treatments were pooled. Separated analysis for each growth stage demonstrated that estimates of leaf N concentration by SPAD was improved as plants grew (correlation coefficients of leaf N and SPAD were 0.08, 0.31 and 0.63 at vegetative, first-pod and late pod, respectively). Similarly, SPAD readings were correlated with SLN values at late pod only, and no correlation was detected for SLW with SPAD values at any stage (Table 8.5). Separated analysis for each environment in Saskatoon (GD and SKA) showed that the greatest correlation coefficient of SPAD and leaf N concentration occurred at GD, whereas the severe drought at SKA changed the association of leaf N-SPAD values, likely due increased leaf thickness.

Table 8.5 Correlation coefficient of SPAD values, SLN and SLW for different lentil cultivars grown in different environments and under different N regimes at three growth stages of vegetative growth (veg), first-pod (FP) and late-pod (LP).

| | SPAD | | | $SPAD_{adj}$ | | |
|---|---|---|---|---|---|---|
| | Veg | FP | LP | Veg | FP | LP |
| **Leaf N (%)** | -- | 0.36 | 0.66 | 0.50 | 0.45 | 0.76 |
| **SLN** | -- | 0.32 | 0.51 | -0.51 | -0.54 | -- |
| **SLW** | -- | -- | -- | -0.75 | -0.74 | - 0.66 |

When SPAD values were adjusted for leaf thickness, *i.e.* SPAD readings divided by SLW, correlation coefficients of the adjusted SPAD values ($SPAD_{adj}$) with leaf N was slightly improved. For example, $SPAD_{adj}$ could explain 50%, 51% and 75% of variations in leaf N concentration, SLN and SLW at vegetative growth, respectively; whereas no strong correlation was detected for SPAD and these variables (Table 8.5). Similarly, SPAD values were not correlated with yield at any stage, but correlation coefficient of $SPAD_{adj}$ and yield were 0.32, 0.47 and 0.50 at vegetative growth, first-pod and late-pod, respectively (data not presented).

### 8.4.10 Leaf N predication

Regression lines predicted leaf N concentration and SLN based on different combinations of SPAD (or $SPAD_{adj}$) values, SLW and growth stages as independent variables. Due to non-significant coefficient of determination ($R^2$) of regression lines at the vegetative growth and first-pod stages, presented results are limited to the late-pod observations only. But the entire data set was used in regression analysis when sampling time (growth stage) was an independent variable (Table 8.6).

SPAD values could not solely provide accurate predicts for Leaf N concentration ($R^2$=0.43). By including leaf thickness in the regression analysis as an independent variable, the accuracy of the model was increased to 0.56, similar to the $R^2$ for linear regression of $SPAD_{adj}$ against leaf N (Table 8.6). These associations further demonstrated that association of SPAD-leaf N concentration was affected by leaf thickness. Combining the entire data set from all growth stages resulted in low $R^2$ for estimated leaf N by SPAD (or $SPAD_{adj}$), SLW and growth stages as independent variables (Table 8.6). Compared to the leaf N-SPAD models, predicted SLN by SPAD or $SPAD_{adj}$ and using the entire data set resulted in improved $R^2$ (Table 8.6). These regression equations demonstrated that by considering leaf thickness SPAD values could reliably predict leaf N concentration of lentil after pod set. For predicting leaf N concentration by SPAD values, both leaf thickness and leaf area must be used as independent variables.

Table 8.6 Fitted lines for estimating leaf N concentration and SLN (g N $m^{-2}$ leaf) using different combinations of SPAD readings (or $SPAD_{adj}$), SLW (g DW $m^{-2}$ leaf) and growth stages (Stage) as independent variables. The lines were fitted for observation at late-pod stage only, when sampling time was absent from the equations, or for the entire data set (3 stages), when growth stage was an element of the regression line.

| Dependent variable | Growth stage | Linear regression equation (Independent variables) | Coefficient of determination |
|---|---|---|---|
| **Leaf N concentration** (%) | Late-pod | 1.787450 +0.06172 (SPAD)<br>3.29188 + 0.05577 (SPAD) – 0.02774 (SLW) | $R^2$= 0.43<br>$R^2$= 0.56 |
| | 3 Stages†† | 3.7019282 + 0.04746 (SPAD) - 0.01824 (SLW) – 0.018239 (Stage) | $R^2$= 0.41 |
| | Late-pod | 1.98964+2.48948 ($SPAD_{adj}$) †<br>1.71367 + 2.60901 ($SPAD_{adj}$) + 0.00413 (SLW) | $R^2$= 0.57<br>$R^2$= 0.58 |
| | 3 Stages | 2.41266+ 1.94131 ($SPAD_{adj}$) + 0.01150 (SLW) -0.18684(Stage) | $R^2$= 0.40 |
| **SLN** (g N $m^{-2}$ leaf) | Late-pod | †<br>-0.063975 + 0.02622 (SPAD) + 0.02048 (SLW) | <br>$R^2$= 0.59 |
| | 3 Stages | -0.25814 + 0.02529 (SPAD) + 0.03116 (SLW) – 0.08664 (Stage) | $R^2$= 0.70 |
| | Late-pod | †<br>-0.06771 + 1.13343 ($SPAD_{adj}$) + 0.034057 (SLW) | $R^2$= 0.01<br>$R^2$= 0.55 |
| | 3 Stages | -0.07804 + 0.92037($SPAD_{adj}$) + 0.04547 (SLW) – 0.08779 (Stage) | $R^2$= 0.67 |

†Estimated line was not presented due to small $R^2$
†† Three growth stages of vegetative growth, first-pod and late-pod were arbitrary called 1, 2 and 3, respectively.

### 8.4.11 Non-linear regression, using a neural network model

For non-linear regression analysis by a neural networks (NN) model, data from different environments and three growth stages were combined and analyzed. Then, precision of the estimated lines were tested by correlating the predicted values by NN against the actual observations. Results showed that the NN model can produce more accurate estimates for leaf N concentration and SLN than linear regression for all combinations of SPAD (or $SPAD_{adj}$), SLW and growth stage as independent variables. Different from the linier regression lines, fitted lines by the NN were identical for leaf N and SLN (Table 8.7).

Table 8.7 Correlation coefficient (r) between prediction line by the NN model and actual observations. The predicted line was estimated by different combinations of combinations of SPAD (or $SPAD_{adj}$), SLW and growth stages (Stage) as independent variables. Data are pooled for three growth stages.

| **Output (y)** | **Input x (Independent variables)** | **Accuracy of the predicted line (r)†** |
|---|---|---|
| **Leaf N concentration** (%) | SPAD | 0.60 |
| | SPAD, Growth stage | 0.73 |
| | SPAD, Growth stage, SLW | 0.81 |
| | $SPAD_{adj}$ | 0.73 |
| | $SPAD_{adj}$, Growth stage | 0.73 |
| | $SPAD_{adj}$, Growth stage, SLW | 0.83 |
| **SLN** (g N $m^{-2}$ leaf) | SPAD | 0.52 |
| | SPAD, Growth stage | 0.60 |
| | SPAD, Growth stage, SLW | 0.81 |
| | $SPAD_{adj}$ | 0.75 |
| | $SPAD_{adj}$, Growth stage | 0.81 |
| | $SPAD_{adj}$, Growth stage, SLW | 0.88 |

† Accuracy of predicted model was estimated by coefficient correlations of the estimated values by predicted lines against actual observations

Results of the NN model analysis demonstrated that leaf thickness measurements could be omitted for estimating leaf N concentration by SPAD values. In this model, accuracy of prediction was improved only 8%, when SLW was included to the model as an independent variable. In the NN model, SPAD values estimated 73% variation of leaf N concentration, when plant age (growth stage) was considered. The NN line was ~ 17% more accurate than estimation by the linier model. Due to difficulties toward SLW measurements, the NN model can be used for rapid estimate of leaf N by SPAD chlorophyll meter and the NN model in the field. The nonlinear curves for SPAD (or $SPAD_{adj}$) were slightly improved when data were limited to the last measurement at late-pod. Correlation coefficient of estimated values by the NN against actual observations was improved by 32% and 12% by using the late-pod SPAD and $SPAD_{adj}$ values, respectively compared to using the entire data set (Fig 8.5).

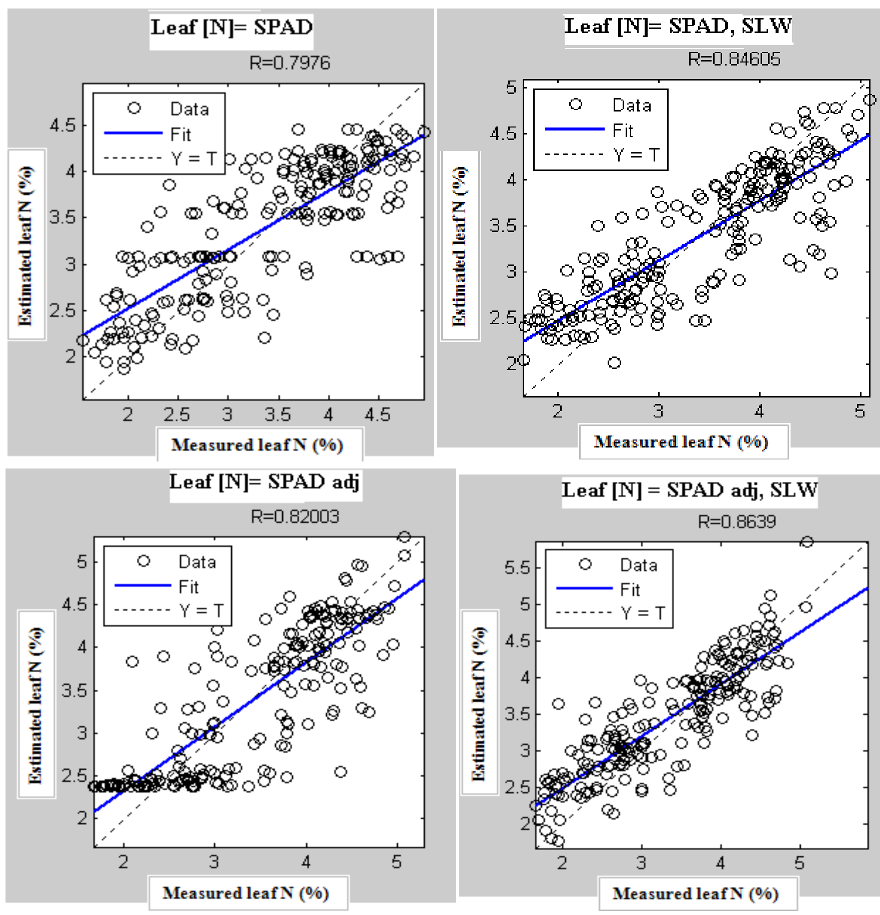


Figure 8.5 Correlation coefficient between predicted leaf N concentration (vertical) by NN model and actual measurements (horizontal), using SPAD or $SPAD_{adj}$ as independent variable. Unites of SLW and SLN are g DW $m^{-2}$ leaf and g N $m^{-2}$ leaf, respectively.

## 8.5 Discussion

### 8.5.1 Leaf N and environment

Leaf N concentration after pod-set can be used as an estimate of the whole lentil N status and yield in lentil. For example, when lentil yield was limited under LN in 2006, leaf N concentration, SLN and SLW were also low. At GD, fertilized lentil had greater leaf N concentration than non-fertilized plots at first-pod, but plant N content was similar amongst the treatments, demonstrating that leaf N measurements at the plant apex better represented availability of N to lentil. Again, the uppermost leaves after flowering did not have a strong correlation with N concentration of the entire leaf biomass (Table 8.4). Kashiwagi et al., (2006) indicated that top leaves better responded to limitation, including N shortage, than the entire canopy.

Changes in leaf N status by growth stage is attributed to lowered light penetration to the canopy, should be considered in leaf N concentration measurements (Lemaire et al., 1991; Gordon et al., 1982). Impact of leaf expansion and leaf thickness on leaf N concentration is more important in dicots because light penetration to the base of canopy is decreased with the growing season (Lemaire et al., 1991). However, measurements were limited to the top of canopy so leaf photosynthesis and leaf N were not affected by light intensity. In this scenario, varied leaf N concentration along with plant growth may result from varied leaf area and leaf thickness (Koch et al., 1988) as well as competition of pods with leaf for N (Gastal and Lemaire, 2002).

Variation in leaf thickness extended toward plant maturity in both droughts (SKA) and wet conditions (IH07), indicating cultivar variability for leaf maintenance under drought (SKA) or leaf area expansion with more available water (IH07). Gastal and Lemaire (2002) suggested that variation in leaf N concentration can be reduced by converting leaf N concentration to SLN. Leaf thickness (SLW) has been used as an indicator of plant hardiness in stressful environments (Magliette et al., 1995). In the current study, despite similar early-season rainfall at GD and SKA (Fig 4.1), lentil had lower leaf N concentration and thicker leaves at SKA than at GD at vegetative growth, more likely because of less available N in this environment (Table 4.1). Comparison of lentil under varied conditions suggested that water availability was strong factor on leaf N concentration along with N availability.

Although overall correlation between $N_2$ fixation and leaf N concentration was not strong, leaf N concentration followed similar pattern as total fixed N by lentil under different N fertility treatments. At SKA, lentil at mid-pod had greater $N_2$ fixation in the inoculant than in other treatments (Fig 4.1, Chapter 4), as did greater leaf N concentration. However, due to the drought conditions at SKA, the association of leaf N concentration and $N_2$ fixation disappeared at late season. In this environment, both severe N deficiency (in control) and leaf expansion (in the fertilizer treatment) resulted in low leaf N concentration compared to lentil in the inoculant treatment. In soybean, greater SPAD readings and SLN of nodulated soybean resulted from higher $N_2$ fixation, compared to non-nodulated genotypes (Sheshshayee et al., 2006).

Lack of variation in the leaf parameters among different cultivars in either experiment was attributed to variation in cultivars phenology and sampling date. Leaf sampling was conducted at average growth stages across the experimental plots in each environment; therefore, variation in the phenology of different cultivars may alter differences of cultivars for the leaf characteristics. At late-pod, CDC Blaze had less leaf N concentration and greater SLW compared to other cultivars, possibly because of earlier maturity. In sorghum, stay green cultivars had greater SLN than early maturing, early leaf senescence cultivars (Borrell et al., 2001).

### 8.4.2 Leaf N versus plant N

Leaf N concentration and SLN were not correlated at vegetative growth stage, but they were positively associated after first-pod, possibly due to varied leaf area (Campbell et al., 1990) and N remobilization due to the onset of competition between pods and leaves for N (Ono et al., 1996; Aerts, 1990). The varied association of leaf N concentration and SLN with yield in table 8.4 was explained by positive effects of prolonged photosynthesis under drought and negative effects of expanded leaf during maximum growth (Nageswara Rao et al., 2001).

The strong association of leaf N concentration, measured at the top of canopy, with N concentration of the entire leaf biomass at vegetative growth (r= 0.64), disappeared later. This variation demonstrated that N status of newly formed leaves at the top of the canopy differed from the N status within the entire leaf biomass. Lentil is an indeterminate crop and continuous leaf production after flowering and pod set may compete with other organs for N.

### 8.4.3 Prediction of leaf N by SPAD

Association of SPAD with leaf N concentration was increased as plants grew and maximized at late-pod sate (Table 8.5). Similar results were found for groundnut (*Arachis hypogeal* L.), which SPAD-SLN association was maximized 60 days after seeding (Nigam and Aruna, 2008). Likewise, adjustment of SPAD readings for leaf thickness improved the association of SPAD with both leaf N concentration and SLN (Table 8.5, 8.6 and 8.7). Chapman and Barreto, (1997), improved the association of SPAD-lean N by more repeated measurements per leaf and by adjusting SPAD readings for SLW.

Smaller correlation coefficient of SPAD-leaf N in the current study, compared to experiments on other crops (Esfahani et al., 2008; Magliette et al., 1995) may associate with the ability of lentil for $N_2$ fixation. Sufficient N fixation can reduce variation of leaf N as well as variations in SPAD readings. Substantial variation among cultivar phenology, which was not carefully considered in sampling time, could lower the correlation coefficient of SPAD-leaf N concentration. Also, varied conditions of the experiment affected on the SPAD-leaf N concentration relationship. For example, at SKA, variation of SPAD matched the variation in leaf N concentration and SLN. However, SPAD readings failed to explain total plant N and yield, more likely due to the effect of drought on leaf thickness, N remobilization and N partitioning among within lentil plants (Magliette et al., 1995). Finally, strong correlation of predicted values by non-linear NN model and actual observations (Table 8.7) demonstrated that lentil leaf N concentration was affected by various factors, not by N availability only. The NN model could apply weights or importance to each observation into the data set. Such weighting will vary and account for different cultivars, growth stages and treatment effects.

## 8.6 Conclusion

Results of this study demonstrated that lentil leaf N concentration represented soil and plant N status. Similarly, SPAD values, especially when adjusted for SLW, represented plant and soil N status and yield of lentil. SPAD values could be used to predicted leaf N concentration at various growth stages of lentil, with the strongest correlation of SPAD- leaf N concentration at the late-pod stage, when pods competed with leaf for N. Linear regression analysis showed that lentil leaf N concentration could be predicted by combination of SPAD values and SLW in both linear and NN models. When a measurement for the entire growing season is desired, SPAD values could provide better prediction of SLN than leaf N concentration. Compared to linear regression, the NN model was accurate enough to estimate leaf N concentration without correcting the SPAD values for SLW. Such an advantage may be used for rapid measurement of leaf N concentration in field. For further studies, limited cultivars and repeated observations during the growing season may improve the association of leaf N concentration-SPAD values.

# 9.0 General discussion

Lentil growth and yield were more strongly affected by the environment than other variables. For example, the coefficient of variation among plots and environments at maturity in the N fertility study was 65% for *%Ndfa* and 40% for final biomass and yield, indicating large variability. Similarly, lentil yield dramatically fluctuated among the three years of study in the no-till experiment. In the greenhouse experiment, both soil media varied in water holding capacity and initial N content and resulted in different amounts of growth and yield of lentil. In the field studies, environmental effects were associated with rainfall and temperature distribution within a growing season. Noticeably greater final biomass of lentil at GD than at SKA and IH in the fertility study was associated with simultaneous heat and water availability during the period of maximum growth (Fig. 9.1).

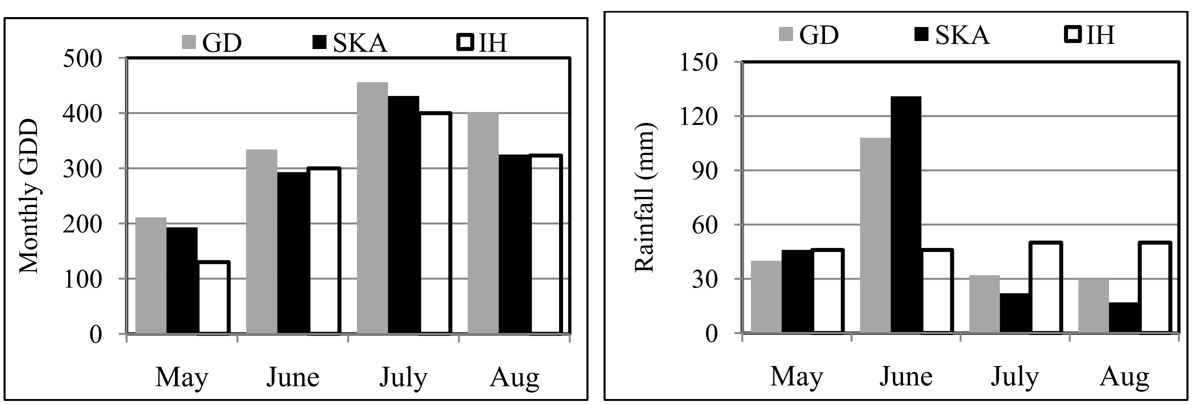


Figure 9.1 Monthly accumulated GDD (Tb=5C) and rainfall in three environments of GD, SKA and IH.

The general conclusions and implications from the findings are discussed under the seven hypotheses developed in the introduction.

I. *Nitrogen fertilizer will reduce $N_2$ fixation of lentil in symbiosis with soil indigenous rhizobia. The lowered $N_2$ fixation will reduce plant N content and, thereby, induce earlier maturity, compared to the inoculated lentil.*

Lentil $N_2$ fixation was occasionally affected by the fertility treatments, but changes in plant N status did not alter days to maturity in any environment. Instead, lentil maturity was governed by seasonal rainfall distribution. Lentil matured earlier in dry conditions of SKA compared to GD and IH, where rainfall continued until late season (Table 4.2). The earlier maturity under drought conditions substantially reduced plant DW, plant N content (g N $m^{-2}$) and seed yield of lentil. Findings of this experiment demonstrated that the N fertilizer treatment reduced lentil $N_2$ fixation by flowering across three environments, and until maturity in the dry conditions of SKA. However, 50 kg N $ha^{-1}$ did not further change total plant N content at maturity.

At SKA, total fixed $N_2$ by lentil in the inoculated and control treatments were similar, but lentil accumulated more total plant N in the inoculated treatment than in the control by maturity. The lowered $N_2$ fixation of lentil in the N fertilizer treatment at SKA (Table 4.7) may be associated with a combination of effects of soil N content, soil cultivation background and within-season rainfall distribution.

In contrast, non-inoculated lentil did not support maximum biomass and N accumulation at IH, where lentil received more rainfall during the period of maximum growth. The lowered $N_2$ fixation of the untreated control in this environment suggested that recommendations for application of rhizobia inoculants can increase growth and yield of lentil under different conditions.

II. *Different N fertility treatments will influence time of the availability of N supply to the plant. More available N to fertilized lentil at early growth will increase pre-flowering dry weight (DW); whereas post-flowering growth will be promoted in the inoculated lentil. This variability will impact partitioning of DW and N within lentil plants.*

Findings of the fertility treatments study at GD and SKA allowed me to reject the above hypothesis, because applying 50 kg N fertilizer ha$^{-1}$ did not enhance early or mid season growth (flowering and podding stage) leaf and stem DW and N content (g N m$^{-2}$), compared to the other two treatments. Furthermore, late season N availability (via fixation or uptake) increased pod DW and pod N, rather than improving DW and N content of stem and leaf (Fig 5.2). Pods contained the greatest portion of DW (75% of plant DW) and N (80% of plant N) by maturity (Fig. 5.1). Such a sizeable proportion, which indicates a great impact of sink demand on DW and N partitioning, is less likely to be affected by N availability. Under suitable conditions of temperature, water and N, pods can strongly compete with stem and leaf for carbohydrate and N.

Greater stem DW and N content of lentil under fertilizer treatment compared to other treatments at SKA could be associated with drought, which halted lentil growth and development. In a normal longer growing season, continuous $N_2$ fixation, more N mineralization and greater N remobilization from stems to seeds would result in similar stem DW and N content across the N fertility treatments at SKA, as observed at GD.

Lack of variation in leaf and stem DW and N content in response to the fertility treatments at different stages of growth may be associated with plant-soil interactions. Factors involved would include: a low growth rate and low N demand of lentil prior to flowering; N fertilizer movement to deeper depths; continuous N mineralization and establishment of $N_2$ fixation by podding; and strong competition of pod with leaf and stem for carbohydrate and N during reproductive growth. Similar leaf and stem DW and N content were seen across the three fertility treatments. At both environments of GD and SKA, total June rainfall was almost twice the average long-term June rainfall in Saskatoon (Table 4.2).

III. *Response of different lentil cultivars to the N fertility treatments will be different. Available cultivars to Saskatchewan farmers differ in days to maturity, seed size and therefore seed N demands. Their $N_2$ fixation ability and potential for biomass and N accumulation will also vary. Less response to the three N fertility treatments is expected in cultivars with small biomass and low yield potential.*

The study results were insufficient evidence to reject the above hypothesis. In general, small seeded cultivars tended to produce plants with smaller biomass by maturity than large-seeded cultivars. CDC Blaze, CDC Viceroy, and occasionally CDC Rouleau accumulated less DW than other cultivars. Although early maturing cultivars are suggested to perform better in the short growing seasons of the northern Great Plains, maximum yield in both Dark Brown (Saskatoon) and Black Soil Zones (Indian Head) belonged to CDC Plato and other large-seeded cultivars which matured later than the small-seeded ones. CDC Blaze, CDC Milestone and CDC Viceroy matured before CDC Plato, CDC Sedley and CDC Greenland in all three environments, but did not produce as much yield as the late matured cultivars. Variation of cultivars for yield was also associated with their ability for $N_2$ fixation and N accumulation.

IV. *High soil N content due to long-term no tillage will increase plant DW, N content and yield and extend the period of seeding to maturity. Lentil grown in long-term NT will have smaller HI compared to lentil grown in short-term NT. Response of varied cultivars to the duration of NT will differ because cultivars vary in biomass, N accumulation and seed yield.*

Based on the no-till study results at Indian Head, the above hypothesis was rejected. Greater N in the long-term NT (Table 6.1) did not improve yield, nor delay maturity via stimulated vegetative growth, when all three years data were considered (table 6.2). Surprisingly, short-term NT (SN) resulted in greater DW, N content and yield of lentil compared to long-term NT (LN) in 2006. In this year, temperature was appropriate for good growing conditions throughout the entire growing season, and high rainfall during the period of maximum growth was followed by a mild-terminal drought (Fig 6.1). Compared to 2007 and 2008, years with cooler growing seasons and continuous rainfall toward plant maturity, lentil yield was maximized in 2006. Days to maturity remained independent from the NT duration throughout the entire study, including 2006, and plants were always forced to dry to harvest maturity by use of desiccants.

Although HI negatively impacted by plant N content (Table 6.5), the smaller yield of LN lentil than SN lentil in 2006 was associated with smaller plant N concentration and plant N content (table 6.3), suggesting minor effects of indeterminate growth on HI and yield reduction. Furthermore, stimulated vegetative growth in response to more plant N in SN (2006) did not reduce lentil yield. A cultivar with more indeterminate growth (CDC Sedley) produced smaller yield than others due to lack of synchronized DW and N accumulation, i.e. smaller N concentration at maturity. The only significant effect of NT duration on lentil growth and yield, which was observed in 2006, could be explained by the pattern of temperature and rainfall distribution during the growing season.

Data averaged over three years showed that only CDC Robin produced greater yield, and accumulated more DW and N content in SN than in LN (Fig 6.2). The only large-seeded cultivar in the no-till study, CDC Sedley, along with CDC Vantage (medium green-seeded) accumulated the greatest biomass, but produced fewer yields than the other three cultivars (Table 6.6). However, cultivars did not differ for days to maturity. Occasionally the response of cultivars to the NT duration could be explained by their different $N_2$ fixation values (2008). Greater available N in the LN could reduce %*Ndfa* during the entire season, compared to SN. This variation existed when lentil %*Ndfa* was estimated in 2008.

V. *Response of lentil to N fertilizer will be reduced with more years spent under no-till, because of more available N in long-term than in short-term NT.*

The 5$^{th}$ hypothesis was rejected, and lentil DW and N accumulation as well as yield was independent from NT duration and N fertilizer rates. Lack of consistent response of cultivar CDC Sedley to the 0 to 60 kg N ha$^{-1}$ treatments suggested that soil and atmospheric N was sufficient to support growth and yield of this cultivar in absence of additional N. Comparisons of yield, DW, N content and seed N concentration of CDC Sedley under two tillage systems and four N fertilizer rates suggested that any possible effect of NT duration on yield of this cultivar was more likely associated with $N_2$ fixation. Although not always significant, maximum performance of CDC Sedley was observed under 30 and 15 kg N $ha^{-1}$, which may inhibit $N_2$ fixation less than the 60 kg N $ha^{-1}$ treatment (Fig 6.3, Table 6.8).

VI. *Application of N during the reproductive growth phase will reduce competition between seed and other plant organs for N, and will increase leaf longevity, days to maturity and yield. Response of different cultivars to the post-flowering N application will be different.*

Results of the greenhouse study did not provide sufficient proof to reject the $6^{th}$ hypothesis. Improved lentil yield via applying post-flowering N fertilizer demonstrated that lentil N uptake continued until late reproductive growth. The two post-flowering N treatments, N until podding and N until maturity, increased lentil yield compared to the inoculated lentil, when $N_2$ fixation was restricted in the second soil medium (Table 7.3). Unlike the field experiments, availability of N affected days to maturity of lentil in the greenhouse study. Availability of N via fixation or N fertilizer extended the period of flowering to maturity and increased lentil yield (Fig. 7.1). This variation among the five cultivars in this study was also associated with variations in days to flowering. CDC Rouleau flowered almost 10 days later, and fixed more $N_2$ prior to flowering, compared to other cultivars. Therefore, yield of CDC Rouleau in the inoculated treatment was identical to its yield in the post-flowering N application treatments.

VI. *Leaf N status will represent the entire plant N status in response to varied environments and N availability. Leaf chlorophyll content, estimated by the SPAD chlorophyll meter, will provide accurate estimation and prediction of leaf N concentration.*

Leaf N concentration closely reflected plant N status; however, the results varied by environment and growth stage. Early-season leaf concentration represented soil N availability, and clearly showed variability of soil N due to varied N treatment applications. As plants grew, leaf thickness affected leaf N concentration, with the greatest effect of the leaf thickness in drought. Interestingly, greater leaf N concentration was seen in plants receiving inoculant rhizobium under drought, where $N_2$ fixation was also maximized by the inoculant treatment, demonstrated that the newly formed leaves on the top of canopy take the more recent fixed $N_2$ and possibly compete with pods.

The SPAD chlorophyll meter could predict about 66% of variation in leaf N concentration at late-pod, when plants were turning yellow. By adjusting the SPAD values for leaf thickness, the correlation coefficient was improved by 0.76 (Table 8.5). A non-linear regression line, produced by a NN model, could predict up to 83% of variation in leaf N concentration (Table 8.7).

**Key findings of the project**

- *Lentil maturity was independent of plant N status across different environments and weather conditions.*
- *Lentil yield was associated with plant DW and N content; therefore, any manipulation of soil available N for maturity induction will affect lentil yield.*
- *Final plant $N_2$ fixation was not affected by the 50 kg N $ha^{-1}$ under normal conditions of water and temperature, suggesting more N fertilizer is required to disturb $N_2$ fixation of lentil. However, early season $N_2$ fixation was strongly inhibited by the N fertilizer treatment.*
- *Inoculation of lentil with rhizobia was more effective in the cool-humid environment of IH where lentil performed poorly in the non-treated compared to lentil performance in drought conditions of SKA, where control lentil $N_2$ fixation was similar for both the inoculated and control treatments.*

- *Under favorable conditions of water, continuous leaf and stem production of lentil did not compete with yield, because they carried only a very small portion of plant N by maturity. Results showed that the contribution of stems to yield was smaller than the contribution of leaf; hence, yield improvement via selecting cultivars with greater stem DW and N content is likely.*
- *Duration of no tillage did not affect lentil growth and yield; however, inhibited $N_2$ fixation by accumulated N in the long-term NT was possible. From results of this study, I highly recommend considering soil temperature and residual herbicide effects on lentil $N_2$ fixation and yield when duration of NT on pulse yield is studied.*
- *Comparisons of different cultivars in three major experiments did not support the superiority of early maturing cultivars over late maturing cultivars. However, yield of late maturing cultivars, like CDC Sedley, can be limited in cooler areas. Varied response of cultivars under variable conditions of temperature and rainfall was independent from their days to maturity.*
- *SPAD chlorophyll readings can provide reasonable estimates of soil-plant N status at early season and plant N conditions at late-season.*

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**Appendix A:** Lentil biomass (DW), N concentration (N%), total N content, seed yield, harvest index and days to maturity of lentil cultivars under varied N fertilizer regimes in two soil media (M1 and M2) in the greenhouse.

| Cultivar | Treatment | Plant DW at maturity | | Plant N concentration at maturity | | Plant N content at maturity | | Seed yield | | Harvest Index | | Days to maturity | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | g plant$^{-1}$ | | % | | mg plant$^{-1}$ | | g plant$^{-1}$ | | % | | Days from seeding | |
| | | M1 | M2 | M1 | M2 | M1 | M2 | M1 | M2 | M1 | M2 | M1 | M2 |
| **CDC Blaze** | **NI** (Inoculant No-N) | $4.1^{defg}$ | $3.0^{efg}$ | $2.7^{ef}$ | $3.0^{ef}$ | $114^{def}$ | $89^{hi}$ | $1.2^{cdefg}$ | $0.9^{fg}$ | $27^{cdefg}$ | $31^{efg}$ | $87^{cdef}$ | $70^{defg}$ |
| | **NF** (N until flowering) | $3.7^{efgh}$ | $1.5^{h}$ | $2.5^{fgh}$ | $2.3^{g}$ | $91^{efg}$ | $36^{k}$ | $1.3^{bcdefg}$ | $0.7^{fg}$ | $36^{abcd}$ | $43^{abcd}$ | $81^{def}$ | $69^{defg}$ |
| | **NP** (N until mid-pod) | $4.5^{cdef}$ | $4.7^{bc}$ | $3.4^{abc}$ | $4.0^{bc}$ | $155^{bcd}$ | $190^{de}$ | $0.9^{efg}$ | $2.0^{bcd}$ | $21^{g}$ | $42^{abcde}$ | $98^{abcd}$ | $89^{bcde}$ |
| | **NM** (N until maturity ) | $5.4^{bcd}$ | $2.6^{fgh}$ | $3.8^{a}$ | $4.7^{a}$ | $201^{abc}$ | $120^{fgh}$ | $2.0^{abc}$ | $1.2^{efg}$ | $36^{abc}$ | $46^{ab}$ | $110^{abc}$ | $73^{defg}$ |
| **CDC Rouleau** | **NI** (Inoculant No-N) | $4.2^{defg}$ | $4.2^{bcd}$ | $2.6^{efg}$ | $3.1^{e}$ | $111^{def}$ | $134^{f}$ | $1.4^{bcdefg}$ | $1.9^{cde}$ | $31^{abcdef}$ | $44^{abcd}$ | $103^{abcd}$ | $92^{bcd}$ |
| | **NF** (N until flowering) | $3.3^{fgh}$ | $4.7^{bc}$ | $1.9^{i}$ | $2.7^{fg}$ | $64^{fg}$ | $129^{fg}$ | $0.9^{efg}$ | $2.4^{abc}$ | $28^{bcdefg}$ | $50^{a}$ | $92^{bcdef}$ | $125^{a}$ |
| | **NP** (N until mid-pod) | $6.2^{ab}$ | $6.3^{a}$ | $3.2^{cd}$ | $3.6^{d}$ | $200^{abc}$ | $224^{cd}$ | $1.8^{abcde}$ | $2.5^{abc}$ | $28^{bcdefg}$ | $39^{abcde}$ | $100^{abcd}$ | $109^{abc}$ |
| | **NM** (N until maturity ) | $5.7^{abc}$ | $7.3^{a}$ | $3.4^{abc}$ | $4.0^{bc}$ | $198^{abc}$ | $293^{a}$ | $1.6^{bcdef}$ | $2.7^{abc}$ | $27^{cdefg}$ | $38^{bcdef}$ | $107^{abcd}$ | $125^{a}$ |
| **CDC Milestone** | **NI** (Inoculant No-N) | $3.5^{efgh}$ | $2.6^{fgh}$ | $2.7^{efg}$ | $3.0^{ef}$ | $93^{efg}$ | $77^{ij}$ | $1.0^{defg}$ | $0.9^{fg}$ | $29^{bcdefg}$ | $34^{cdefg}$ | $67^{f}$ | $63^{fg}$ |
| | **NF** (N until flowering) | $3.2^{fgh}$ | $1.6^{h}$ | $2.3^{ghi}$ | $2.4^{g}$ | $74^{fg}$ | $40^{jk}$ | $1.2^{cdefg}$ | $0.8^{fg}$ | $36^{abcd}$ | $50^{a}$ | $69^{ef}$ | $63^{g}$ |
| | **NP** (N until mid-pod) | $6.1^{ab}$ | $5.2^{b}$ | $3.5^{abc}$ | $4.0^{bc}$ | $216^{a}$ | $209^{cd}$ | $2.5^{a}$ | $2.3^{abc}$ | $40^{a}$ | $45^{ab}$ | $94^{bcde}$ | $112^{abc}$ |
| | **NM** (N until maturity ) | $5.8^{abc}$ | $2.9^{efg}$ | $3.7^{a}$ | $4.3^{b}$ | $215^{a}$ | $122^{fgh}$ | $2.0^{abc}$ | $1.1^{fg}$ | $34^{abcde}$ | $37^{bcdef}$ | $95^{abcde}$ | $87^{cdef}$ |
| **CDC Greenland** | **NI** (Inoculant No-N) | $4.6^{cde}$ | $3.4^{def}$ | $2.9^{de}$ | $2.7^{fg}$ | $137^{de}$ | $93^{ghi}$ | $1.8^{abcd}$ | $0.8^{fg}$ | $38^{ab}$ | $23^{g}$ | $97^{abcd}$ | $80^{defg}$ |
| | **NF** (N until flowering) | $2.7^{h}$ | $3.2^{def}$ | $2.1^{hi}$ | $2.5^{g}$ | $55^{g}$ | $83^{hi}$ | $0.9^{fg}$ | $1.3^{defg}$ | $33^{abcde}$ | $38^{bcde}$ | $69^{ef}$ | $87^{cdefg}$ |
| | **NP** (N until mid-pod) | $6.1^{ab}$ | $3.9^{cde}$ | $3.4^{abc}$ | $4.1^{bc}$ | $211^{a}$ | $160^{ef}$ | $1.7^{abcdef}$ | $1.3^{def}$ | $27^{cdefg}$ | $33^{defg}$ | $107^{abcd}$ | $90^{bcde}$ |
| | **NM** (N until maturity ) | $6.8^{a}$ | $6.6^{a}$ | $3.7^{ab}$ | $4.0^{bc}$ | $249^{a}$ | $268^{ab}$ | $2.1^{ab}$ | $3.1^{a}$ | $31^{abcdefg}$ | $47^{ab}$ | $117^{ab}$ | $125^{a}$ |
| **CDC Sedley** | **NI** (Inoculant No-N) | $5.2^{bcd}$ | $3.3^{def}$ | $3.0^{de}$ | $2.6^{fg}$ | $153^{cd}$ | $87^{hi}$ | $1.3^{bcdefg}$ | $0.9^{fg}$ | $26^{defg}$ | $26^{fg}$ | $121^{a}$ | $66^{efg}$ |
| | **NF** (N until flowering) | $3.1^{gh}$ | $2.0^{gh}$ | $2.1^{hi}$ | $1.9^{h}$ | $67^{fg}$ | $38^{jk}$ | $0.7^{g}$ | $0.5^{g}$ | $21^{fg}$ | $24^{g}$ | $83^{def}$ | $66^{efg}$ |
| | **NP** (N until mid-pod) | $6.4^{ab}$ | $6.4^{a}$ | $3.3^{bcd}$ | $3.7^{cd}$ | $210^{ab}$ | $236^{bc}$ | $1.8^{abcde}$ | $2.8^{ab}$ | $27^{cdefg}$ | $44^{abcd}$ | $102^{abcd}$ | $113^{ab}$ |
| | **NM** (N until maturity ) | $5.7^{abc}$ | $5.1^{b}$ | $3.7^{a}$ | $4.1^{b}$ | $211^{a}$ | $210^{cd}$ | $1.4^{bcdefg}$ | $2.3^{bc}$ | $24^{efg}$ | $45^{abc}$ | $117^{ab}$ | $125^{a}$ |